OBITUARY

PROFESSOR W. T. MACCLEMENT, M.A., D.Sc., LL.D.,

1861-1938

Professor W. T. MacClement, who died in August of last year, was a distinguished member of an older generation of Canadian biologists: great men who with courage, foresight and fine discrimination laid firm foundations for the present and future structure of Canadian biology.

Professor MacClement was primarily a teacher—a master in Ontario collegiates, professor of chemistry in the Armour Institute and for nearly thirty years professor of biology at Queen's. He made a great contribution to science—enthusiastic and well trained students. Many passed through his hands, none failed to profit.

For thirty years he took an active interest in the Biological Board. He was associated with Dr. Knight in many of his lobster studies and made significant contributions to the knowledge of marine algae, especially in connection with lobster rearing problems. He was appointed a member of the Biological Board in 1920 and a member of the executive committee in 1926. He continued in active service up to the reorganization and formation of the Fisheries Research Board in 1937. During this long period he gave unsparingly of his time and wise counsel. The present highly efficient state of the Board is in no small measure the result of his quiet insistence on high standards.

The Vice-Principal of Queen's said of him: he was a great teacher, a great biologist and a great gentleman.

The Fisheries Research Board and the research facilities under its administration constitute one of several similar memorials to the distinguished group of which Professor MacClement was an honoured member.

Studies of Fish Spoilage

III. The Trimethylamine Oxide Content of the Muscles of Nova Scotia Fish

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ABSTRACT

Trimethylamine oxide was found in the muscle press juice of all salt water fish examined and in the anadromous fish *Pomolobus pseudoharengus* taken from the sea. Traces were found in *Anguilla* taken from salt water, but none in *Anguilla* from fresh water.

INTRODUCTION

The presence of trimethylamine oxide in dogfish was demonstrated by Suwa (1909). He showed also that bacteria reduce the oxide to trimethylamine and concluded that the particular odour of spoiling sea fish is due to the increase in the amine. Beatty and Gibbons (1937) and Beatty (1938) have shown that, in spoiling cod muscle press juice, trimethylamine is produced only as a result of the action of the bacteria producing the spoilage, and that it is derived from the reduction of trimethylamine oxide.

The literature previous to 1933 has been reviewed by Kutscher and Ackermann (1933). The oxide has been demonstrated by the isolation and analysis of typical salts, in selachians and salt water teleosts, but it was not found in *Anguilla vulgaris, Salmo salar, Perca fluviatilis,* or *Cyprinus carpio.*

Not many quantitative estimations of trimethylamine oxide have been made by the German workers. Kapeller-Adler and Krael (1930) found 0.0167 g. of the oxide and 0.161 g. of trimethylamine per 100 g. of codfish muscle. Since trimethylamine exists in unspoiled fish in very small amounts it is probable that the results obtained by these authors are far from correct. Hoppe Seyler (1933) found 0.3 per cent trimethylamine oxide in the tail muscle of lobster, and 0.05 per cent in crayfish muscle. Cook (1931) analysed muscle tissues of several Canadian fishes, reporting from 0.2 per cent to nearly 0.5 per cent in sea fishes, 0.01 to 0.1 per cent in fresh water fish and anadromous fish. Because of the importance of trimethylamine oxide in studies of fish spoilage, and because of the conflict of evidence as to its occurrence in nature, a survey (not yet completed) has been undertaken as to its occurrence in fresh water and sea fish of Nova Scotia.

ANALYTICAL DATA

Trimethylamine oxide was determined by Lintzel's method (1934). Since the primary purpose of the investigation was to aid in the investigation of fish spoilage, and since previous studies were done mainly on press juice, the trimethylamine oxide content of the muscle press juice rather than of the muscle itself was determined. Analyses of press juice and muscle of *Clupea harengus* showed the press juice to be approximately 19 per cent higher in oxide than the muscle itself. The results of the analyses are shown in table I.

TABLE I. Trimethylamine oxide content of the muscle press juice of Nova Scotia fish.

	Number examined	Trimethylamine oxide (per cent) Maximum	Minimum	Average	
SALT WATER SPECIES					
TELEOSTS					
Gadus callarias (cod)	50	0.78	0.59	0.67	
Melanogrammus aeglefinus (haddock)	8	0.43	0.34	0.38	
Pollachius virens (pollock)	7	0.50	0.44	0.48	
Urophycis sp. (hake)	5	0.98	0.78	0.89	
Clupea harengus (herring)	8	0.59	0.44	0.48	
Scomber scombrus (mackerel)	10	0.29	0.22	0.26	
Pseudopleuronectes americanus (flounder)	6	0.54	0.25	0.38	
Limanda ferruginea (dab)	1				0.418
Glyptocephalus cynoglossus (witch)	6	0.56	0.23	0.31	
ELASMOBRANCHS					
Squalus acanthias (dogfish)	6	1.59	1.25	1.43	
Raja laevis (barndoor skate)	1				1.25
Raja senta (smooth skate)	1				0.53
Raja scabrata (prickly skate)	2				0.27
FRESH WATER SPECIES					
Perca flavescens (yellow perch)	5	0.00			
Ameiurus nebulosus (catfish)	6	0.00			
Catostomus commersonii (sucker)	2	0.00			
FRESH AND SALT WATER SPECIES					
Anguilla rostrata (eel)					
From fresh water	3	0.00			
" brackish water	3	0.001	0.005	0.004	
Pomolobus pseudoharengus (gaspereau) from salt water	3	0.28	0.20	0.25	

DISCUSSION

The table brings out the following facts. Muscles of all fish examined, that live constantly in sea water, contain trimethylamine oxide in appreciable quantities. *Pomolobus pseudoharengus,* which may live in the sea, whence it migrates into fresh water to spawn, likewise contains the oxide while in salt water and about to enter fresh water. The oxide was not found in muscle of fresh water fish nor in eels while in fresh water. The traces in the muscle of eels taken in salt water may be due to that ingested in food.

As to any physiological action of the trimethylamine oxide, the fact that *Anguilla* taken from the sea contains practically no oxide, shows that it is not necessary to all life in salt water. The wide difference in the values found for *Squalus acanthias* and *Raja scabrata,* both Elasmobranchs and living in salt water, would indicate that if the concentration in the muscles is any indication of that found in the circulating fluids, the oxide is not an important substance in the maintenance of osmotic equilibrium.

The fact that *Anguilla* in salt water does contain traces suggests that food may have some bearing on the concentration of the oxide in the muscles, but the fact that *Squalus acanthias* is about 1,500 times higher in oxide than *Anguilla* would suggest either that the mechanisms of elimination are widely different or that food has only a minor influence on the concentration in the muscles.

Grollman (1929) showed that the urine of *Lophius piscatorius* is high in the oxide. It is very improbable that this compound is used as a means of nitrogen elimination.

As for the theory that trimethylamine oxide acts as an oxygen donor, the data are not sufficient to draw any conclusions.

The marked difference between sea fish and fresh water fish points possibly to a different mechanism of spoilage. Trimethylamine is one of the earliest compounds produced during the spoilage of sea fish, and is definitely responsible for some of the spoilage odour. Previously, the development of spoilage, as determined by the production of trimethylamine, was followed only in members of the cod family. Since the oxide is so widely distributed in sea fish, its reduction by spoilage bacteria should be a general reaction, although the great differences in the oxide contents of various species may mean that different spoilage threshold values may be necessary.

SUMMARY

Muscles of all sea fish examined contained trimethylamine oxide.

Trimethylamine oxide was not found in muscles of fresh water fish.

The oxide was present in muscle of *Pomolobus pseudoharengus* that migrates into fresh water to spawn, but was absent in muscle of *Anguilla* while in fresh water.

The data throw no light on the physiological importance of trimethylamine oxide.

The production of trimethylamine by spoilage bacteria is likely to be a general phenomenon during the spoilage of all sea fishes examined.

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Homing Tendency and Age at Maturity of Pink Salmon (*Oncorhynchus gorbuscha*) in British Columbia

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ABSTRACT

Pink salmon fry were marked by the removal of certain fins to ensure later identification as adults. This procedure does not affect the growth or the feeding reactions of the fish. Three experiments were conducted on natural runs at McClinton creek, Masset inlet, B.C. On the basis of the most significant one of these it is concluded that the majority of the fish return to spawn in the stream in which they were hatched. Isolated individuals, in numbers not economically significant, may wander to a distance of 400 miles (645 kilometres). In the case of fry resulting from transplantation experiments from Tlell river, east coast of Graham island, to McClinton creek, there appears no consistent behaviour in regard to "homing". For fry, hatchery-raised and pond-reared, from Vedder river, Swelter creek eggs, no return to the parent stream was reported. All pink salmon mature in the autumn of their second year. Certain incidental checks in growth have been discovered on scales which should not be interpreted as representing a winter.

INTRODUCTION

In 1930 the Biological Board of Canada initiated a programme of investigation into the life history of the pink salmon (*O. gorbuscha*) with a view to obtaining information on the natural propagation of the species which might be of value in controlling its exploitation and conservation. The main feature of the plan involved the installation of special counting fences in McClinton creek, a tributary to Masset inlet, Graham island, Queen Charlotte islands, where records could be obtained of the number of adults which arrived to spawn, the potential egg deposition, and the number of fry which resulted to migrate seaward. Before the findings could be applied in so far as the determination of loss in the marine portion of the life history was concerned, two important questions had to be answered, viz.: At what age do these salmon mature? Do they return to spawn in the same stream which they left as fry?

To produce direct information on these points the procedure of *marking*—the removal of one or more fins from the young fish to ensure their later identification—was utilized, since it had produced reliable data in the case of some of the other species of the genus. The present paper reports the results of the various experiments carried out to date for the pink salmon in the waters off the coast of British Columbia.

Other Pink Salmon Marking Experiments

Within recent years four marking programmes have been carried out on the pink salmon in areas contiguous to British Columbia. In 1927 the Department of Fisheries of the State of Washington marked at Quilcene bay in Hood canal by the removal of the dorsal and right ventral fins, 25,300 pink salmon fingerlings, the progeny of eggs transferred from southeastern Alaska. From the same source and at the same place, 15,000 were marked in 1929 by the removal of the dorsal and left ventral fins. No returns have been recorded (Letter A. E. Einarsen, 1938).

Davidson (1934) has reported upon two experiments. The first of these which Davidson regards as "the first known attempt to mark pink salmon fry at the time they normally migrate from the streams" was carried out in the spring of 1930 at the Duckabush river, a tributary to Hood canal, when 36,000 hatchery-reared fry were marked by removing the dorsal and adipose fins. During the autumn of 1931 ten recoveries were made, eight in the Duckabush river, one in the Dosewallips river four miles (6.4 km.) northward, and one in the Hamma Hamma river nine miles (14.5 km.) southward. The second investigation was made at Snake creek, Olive cove, southeastern Alaska, in the spring of 1931. At that time 50,000 fry from the natural run were marked by clipping the adipose and dorsal fins. In the autumn of 1932 out of a total of 7,944 adult salmon taken in Snake creek, twenty-three were found to lack the above-mentioned fins. On the basis of a direct relationship Davidson calculates that in the complete migration of 10,640 there were fifty-four marked salmon resulting from the experiment of 1931. Although several other streams in the vicinity were examined for the presence of similarly deformed individuals, none were reported. From these returns Davidson concludes that the extent to which pink salmon return to the parent stream to spawn is dependent upon the proximity of other pink salmon streams in the vicinity. If the stream is more or less isolated there is very little tendency to wander to others, but if there are other rivers in close proximity, marked fish may find their way into them. All the evidence indicates that the pink salmon mature and return to spawn at two years of age.

METHODS

Material Handled

Six pink salmon marking experiments have been conducted in British Columbia, five at McClinton creek, Masset inlet, and one at Sweltzer creek draining Cultus lake on the lower Fraser river. At McClinton in three instances fry from the natural downstream migration were handled, while in the other two the young fish were the progeny of eggs transferred from the Tlell river on the east coast of Graham island.

McClinton creek natural runs.

Since it had been discovered that the greatest return in adult pink salmon to McClinton creek was approximately seven per cent of the fry migrants resulting from natural spawning, it was evident that in order to have some assurance of sufficient returns upon which to base sound conclusions, large numbers of fry must

be marked. Accordingly in the spring of 1931, the adipose fin only was removed from 185,557 out of a total migration of approximately five million; in 1933, both ventral fins from 107,949 out of two million three hundred thousand; and in 1935, the adipose and both ventral fins from 85,634 out of twelve and one-half million. During the first experiment (1931), since only one fin was being removed, the marking could be carried on concurrently with the counting throughout the period of the run. In the other two years it was found impossible to mark to any extent during the migration, with the result that over half the samples were taken at the beginning and the end of the run when more time was available. The remainder were selected at random and removed to the near-by eyeing station for retention and feeding. The additional growth thus gained greatly facilitated the marking manipulation.

TLELL RIVER-McCLINTON CREEK TRANSPLANTS.

In 1932 and 1936 there were in the hatchery 877,648 and 505,857 fry respectively resulting from the transfers made from the Tlell river to McClinton creek. Of these in the former year 124,002 were marked by the removal of the adipose and left ventral fins, and in the latter 108,200 by the removal of the adipose and right ventral fins.

SWELTZER CREEK-CULTUS LAKE EXPERIMENT.

In addition to these experiments which were all conducted at McClinton creek, Dr. Foerster found it possible to mark at Cultus lake from July to October, 1932, by amputating both ventral fins, 8,741 fingerlings resulting from eggs taken in the Sweltzer creek, a secondary tributary of the Fraser.

The following table arranged chronologically briefly summarizes the material handled:

Date	Source of material	No. in natural run	Stage at marking	No. marked	Mark used
1931	McClinton creek.....	5,400,000 (approx.)	fry	185,557	Adipose only
1932	Sweltzer creek		fingerlings	8,741	Both ventrals
1932	Tlell r.-McClinton creek transfer	877,648	fry	124,002	Adipose and left ventral
1933	McClinton creek.....	2,260,000 (approx.)	fry	107,949	Both ventrals
1935	McClinton creek.....	12,600,000 (approx.)	fry	85,634	Adipose and both ventrals
1936	Tlell r.-McClinton creek transfer	505,857	fry	108,200	Adipose and right ventral

MARKING PROCEDURE

The actual marking procedure was similar in all the experiments. Small numbers of fry were romoved from the fence pen or the hatchery and placed in shallow trays with marquisette bottoms at either end of a special marking trough

(figure 1-A). Due to the special construction of these trays the water in them was shallow and thus the fish could be easily captured. In the act of marking the operator picked up the fish between the thumb and first finger of the left hand in such a way that the finger and thumb rested over the operculum on either side. The fry thus lay back upwards in the crack of the first joint of the finger. In this position any of the fins could be reached with ease by merely turning the hand. Practice resulted in expertness in catching the fry and constant exposure to the

FIGURE 1. Portion of trough and trays employed in marking pink salmon fry in British Columbia.

water made the fingers so sensitive that the pressure could be gauged to such a fineness as to preclude any harm to the fish.

The marking instrument, a pair of sharp, straight-bladed, nail clippers, was manipulated with the right hand. The desired fin was carefully worked between the blades and removed with a sharp clean cut (figure 2). The fish was then immediately placed in a deep wire basket suspended in the trough between the two trays (figure 1-B). The whole procedure of marking could be completed in approximately fifteen seconds so that the fry were not out of water long enough to suffer any injury.

Collection of Returns

The success or failure of any marking experiments rests almost entirely upon the effort expended in the collection of returns. The common practice has been to announce to the industry in the form of correspondence and posters, of the type shown in figure 3, that returns are expected for which a nominal reward will be paid. Co-operation in assembling such returns is specially requested.

Such a method of collection has evident faults. When the fish are being unloaded at the canneries they come up the elevators at such a rate that it is difficult for a person who is watching carefully all the time to detect fish with

Figure 2. Manipulation in marking pink salmon fry showing position of hands, fry and instrument, when removing right ventral fin.

missing fins. The necessary additional attention is too much to expect from the men who are fully occupied with getting the fish from the boat into the cannery bins. When the fish are being fed into the iron chink at the beginning of the canning process such speed is demanded to keep the operation running that only a cursory glance may be given to each individual. It is perhaps unreasonable to ask those whose main concern is the production of a large pack of high grade product in a very short time, to slow up the processing and thus reduce the pack for the very nominal recompense which is offered for the discovery of marked individuals.

In the year 1934 an improvement on this method was instituted by placing a

man in a representative cannery in each district whose sole duty it was to watch for marked fish as they came up the elevators or as they were being fed into the iron chink. This observer could also ascertain the location and time of capture together with other pertinent data. The efficiency of collection was much higher and the best returns were obtained during that year.

In other years in collaboration with the programmes for the collection of marked sockeye salmon there was an observer stationed on the Fraser river who either remained in one cannery or covered all of the plants as often as possible, constantly requesting a careful inspection for marked fish and paying the rewards.

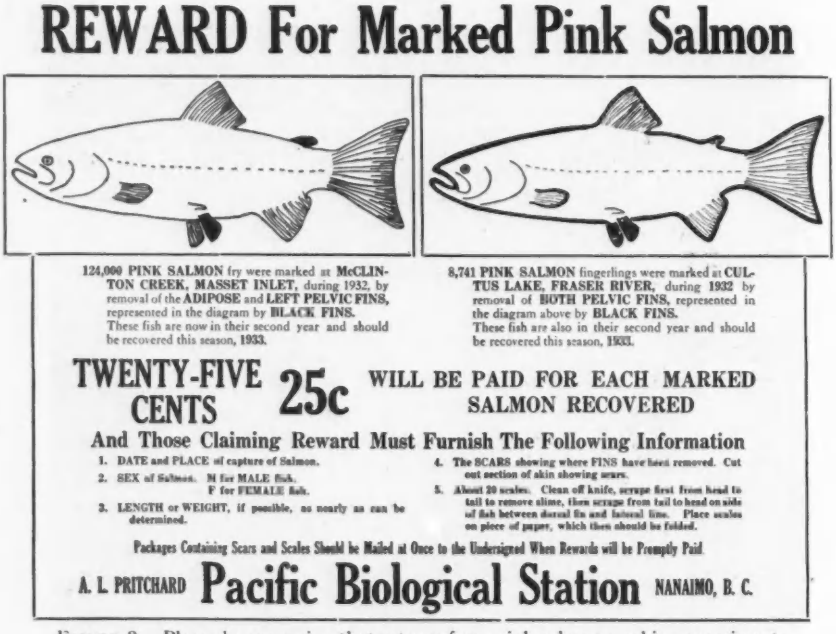

FIGURE 3. Placard announcing that returns from pink salmon marking experiments are expected.

For this reason the efficiency in that area was usually higher than that in the rest of the province where full reliance was placed upon the industry.

At McClinton creek in all years and at the Tlell river in 1933 and 1937, the most perfect system of all was employed in that each fish ascending the streams was dipped from the pens in the fence and examined by an experienced officer.

VALIDITY OF METHODS

EFFECTS OF MARKING

No effect of marking upon the fry could be discovered through the examination of the individuals while they were still held in the wire retaining basket in the trough at the time of marking. Occasionally one or two appeared weak but their

condition could always be traced to the handling during the actual amputation of the fins. These were always removed as samples before the basket was emptied and recorded in the experiment as "Injured at marking".

The retention of marked fry coincidentally with unmarked controls, either in the hatchery troughs or specially designed floating retaining ponds for periods varying from one to four months, failed to reveal any difference either in feeding reactions or survival rate. As a matter of fact, in the summer of 1933 the mortality among the controls was definitely higher than that among the marked fish in the contiguous pen. Thus in so far as the feeding and survival of the fry in a more or less protected environment in the first few months are concerned, marking had no appreciable effect.

Whether the removal of the fins handicaps the fish in any way in its later life and renders it more easily captured by the commercial fishery or by natural enemies is a question upon which there is at present very little decisive information. Harris (1938) has experimented with fish from which the ventral fins have been removed and compared their motion and behaviour with unmarked individuals, with the conclusion that in the lower teleosts amputation does not impair appreciably the equilibrium or powers of locomotion. It is hard to conceive that the removal of the adipose fin would have any more radical effect.

The ratio of return of marked fish to that of unmarked in the adult runs at McClinton creek is pertinent to this subject. A summary is submitted in the following table:

Cycle	Marked			Unmarked		
	Fry migrants	Returning adults		Fry migrants	Returning adults	
	Number	Number	Per cent	Number (approx.)	Number	Per cent
1931-32	185,557	96	0.05	5,200,000	15,504	0.30
1933-34	107,949	2,941	2.72	2,150,000	152,255	7.08
1935-36	85,634	35	0.04	12,500,000	52,277	0.42

In every cycle the proportionate return of unmarked fish is distinctly larger than that of marked. This may be due to a number of causes, amongst which are suggested an efflux of marked fish to other areas coupled with an influx of unmarked fish to McClinton creek, regeneration of the fins which renders the scarred fish unidentifiable, or a greater mortality in marked individuals. On the basis of the data from the one large experiment, that of 1933 to be discussed later, extensive "wandering" would appear to be eliminated. In the section on regeneration (p. 242) it is stated that the small numbers in which fins may grow again are not sufficient to account for the disproportion. The conclusion has been reached above that there is very little visible harmful effect from marking in the fry and fingerlings in fresh water. The removal of the fins also does not appear to affect the ordinary locomotion. That there is a greater relative mortality of marked individuals, however, must be accepted on the basis of the present information. It is possible that another explanation may be forthcoming when more observations are amassed. For

that reason no attempt is made to assign the discrepancy either to natural causes or to the unnatural one of the fishery.

The examination and measurement of the adult fish on their return to McClinton creek in the cycle year has produced interesting information on the condition of those which were marked as compared with those which were normal. Below are submitted for the year 1934 the average lengths and weights together with the probable errors:

Sex	Type	Number	Length (cm.)	Number	Weight (kg.)
Male	Marked	13	57.2±0.693	13	1.99
	Unmarked	417	56.5±0.178	417	1.99
Female	Marked	11	53.3±0.470	11	1.82±.054
	Unmarked	359	52.7±0.076	357	1.70±.008

Since there is no significant difference between the averages of the types either in weight or length, it is evident that marking did not affect the growth of the fish during its sea life. As a matter of fact in almost every case the marked fish are indicated as being slightly larger though the differences are not statistically significant.

Reliability of Original Marks

Since the procedure of marking is somewhat new and strange to individuals not immediately concerned with fisheries research, some difficulty was experienced at the outset in hiring workers sufficiently expert to remove the fins perfectly. Accordingly in order to check any error in manipulation with a view to stimulating those who were secured to reach as nearly to perfection as possible, and, in addition, to allow reasonable interpretation of the peculiarities of the adult fish when they returned, close inspection was maintained throughout. During the spring of 1931 each man at any time was requested to mark a given number of fish and place them in a separate container. These were examined immediately and suggestions advanced as to modifications in manipulation which might rectify further similar errors. In later years when the nucleus of the crew could be chosen from those who had had previous experience, thus reducing somewhat the possibility of faulty marking through complete inexperience, samples were dipped at unstated times from the wire basket around which four men were working.

In the table which follows are presented the results of the examination of all samples of fry and fingerlings obtained during the marking experiments and the retention programmes mentioned previously. Each fish has been assigned to one of three categories on the basis of the condition of the fins supposedly marked, viz. —1,—*cleanly marked*—in which the fins were completely removed, 2,—*partially marked* —in which a portion of any fin was left, and 3,—*unmarked*—in which one or more fins had not been marked at all. In brackets after the numbers of fish under each heading is submitted the percentage which these constitute of the whole sample. In some cases the total of the sections is more than the original number and the percentages add up to more than one hundred. This is due to the

fact, that, if two different fins were *"partially marked"* or *"unmarked"* in the same individual, the fish was assigned to both divisions.

Date	1931	1932	1933	1935	1936
Source of fish	McClinton creek	Tlell r.-McClinton cr. transfer	McClinton creek	McClinton creek	Tlell r.-McClinton cr. transfer
Mark used	Adipose	Adipose and left ventral	Both ventrals	Adipose and both ventrals	Adipose and right ventral
Number	598	88	266	50	135
CLEANLY MARKED	574 (96.0%)	53 (60.2%)	184 (69.2%)	25 (50.0%)	72 (53.3%)
PARTIALLY MARKED					
Ventrals		29 (33.0%)	80 (30.1%)	10 (20.0%)	12 (8.9%)
Adipose	18 (3.0%)	9 (10.2%)		22 (44.0%)	56 (41.5%)
UNMARKED					
Ventrals			2 (0.7%)		
Adipose	6 (1.0%)			1 (2.0%)	

From an examination of the figures above it is apparent that a large number of fry were imperfectly marked. For several reasons such an occurrence is not unexpected and definitely cannot be blamed wholly upon inefficient operators and poor supervision. Pink salmon young at the time of seaward migration are approximately one and one-half inches (3.8 centimetres) in length so that exceptional care and keen eyesight are required for the removal of the fins. It is almost humanly impossible to mark absolutely perfectly such small fish where large numbers must be handled in a short time in order to provide sufficient numbers to ensure a relatively large return. The errors, however, do not negative the value of the results, but the possibility of their occurrence makes it necessary to inspect closely and to compare the variation in the fins of the fry and adults to permit a final reliable interpretation.

When the mark included the removal of only one fin, e.g., the adipose in 1931, an accuracy of 96 per cent was attained. With the addition of more fins and the necessary extra manipulation the precision gradually lessened. In 1932, 1933, and 1936 with two fins involved it was 60.2, 69.2, and 53.3 per cent respectively, while in 1935 with three it amounted to 50 per cent.

Apparently there is no definite trend to the error since in some years the markers were able to remove the adipose fins with the greatest accuracy while in others the ventrals were clipped with the most ease and precision. Undoubtedly weather conditions in so far as they affected the light were the main cause for this inconsistency.

Regeneration

In all marking experiments one important question which must be decided is whether new fins will regenerate in the place of those which have been completely removed. In the present programmes, since the fact had been established that some portions of the fins had not been removed at marking, it was also necessary to know whether the small remnants would grow larger even to the extent of forming a perfect or almost perfect fin in the adult. The examination of the samples taken from the hatchery troughs and retaining ponds up to four months after marking failed to reveal any noticeable change from the condition at the actual time of marking. It was therefore assumed that in the first three or four months no evident regeneration had taken place. In order to check the situation which obtained in this connection during the one and one-half years' residence in the sea, a comparison has been set up between the condition of th fins in the marked adults when they returned to McClinton creek and the fins c samples of marked fry taken at the time of the downstream migration to the sea. The significance of the percentages given in brackets is the same as in the section on "Reliability of original marks".

Cycle	1931–1932		1933–1934		1935–1936	
Date	1931	1932	1933	1934	1935	1936
Mark used	Adipose		Both ventrals		Adipose and both ventrals	
Stage	Fry	Adults	Fry	Adults	Fry	Adults
Number	598	96	266	2,941	50	35
Cleanly marked	574	75	184	1,298	25	8
	(96.0%)	(78.1%)	(69.2%)	(44.1%)	(50.0%)	(22.8%)
Partially marked						
Ventrals			80	1,643	10	25
			(30.1%)	(55.9%)	(20.0%)	(71.4%)
Adipose	18	21			22	7
	(3.0%)	(21.9%)			(44.0%)	(20.0%)

The information summarized in the table demonstrates that between the fry and adult stages percentages of fish which were *cleanly marked* had decreased, while those noted as having *partially marked* fins had increased with only one exception, that of the adipose fins in the 1935-1936 cycle. Some of those fins which were deemed to be cleanly excised must have grown or those partially-cut must have developed further. Thus it is established that regeneration must have occurred during the marine phase of the life history even though it was not indicated in those fish retained for a short while in fresh water. Such a change need not negative the general findings but it does call for caution in the interpretation of the recoveries and in the application of the data therefrom to a determination of ocean mortality. A few pink salmon marked as fry may regenerate their fins and thus become indistinguishable from unmarked adults. In view of the small numbers marked, the phenomenon will not suffice to explain the disproportion of marked and unmarked adults returning to McClinton creek in the various years.

Occurrence of Naturally Deformed Fins

The occurrence of salmon with naturally deformed adipose fins or with this fin entirely lacking through natural causes has been reported upon occasion. Rich and Holmes (1928) recorded the phenomenon for Chinook salmon on the Columbia river. The returns from the sockeye marking made by Chamberlain in Alaska (Chamberlain and Bower 1914) spread over such a long period as to suggest that the adipose might be lacking under natural circumstances. For these reasons a careful inspection was made of the 1930 adult run to McClinton creek. In the 16,000 examined the adipose fins showed no sign of deformity. During the succeeding spring when 185,557 fry were marked, only two were discovered to be without the fin. From samples of the unmarked portion of the run, twelve out of a total of 26,394 were devoid of the fin. Records of the proportion of such naturally deformed fish to the total run were, and still are lacking for other areas. In recent years, however, some presumed adipose fin scars have been returned from Alaska and each year they have consistently appeared in Johnstone strait, at Quathiaski, and in the Fraser river fishery. What percentage these constitute of the total run cannot be determined since the examinations have not been sufficiently thorough. The possibility of the occurrence of such deformities, however, greatly limits the definiteness of any conclusions which might be drawn from experiments in which this fin was used alone.

White and Huntsman (1938), working with Atlantic salmon, have stated that if the adipose fin is cleanly cut, a scar is produced which is quite different from any natural deformity. In the case of their experiment where smolts are being handled which are many times larger than the pink salmon fry, the fins are easily visible and the possibility of error in the marking manipulation is greatly reduced. In the McClinton creek experiments with small fry, remnants of the adipose may be left. It is thus difficult to differentiate in the adults between the results of such an error and that of natural deformity.

Examination of other fins carried out at McClinton creek coincidentally with that of the adipose have shown that in rare cases individuals may occur which lack one or both ventral appendages. In such instances it is usual to find that either a part or all of the pelvic bones are missing. Such natural deformities could be recognized in the scars of the adult fish on close inspection. It is not unusual also for the adult fish to lose part of the pelvic fins apparently as the result of the attack of predators. Fins deformed in this way are often difficult to distinguish from those which are the product of incomplete removal of the fin in the fry.

In experiments involving the marking of fry it is thus preferable to amputate two or more fins, since the chances of more than one fin being absent from a fish in nature, and still unrecognizable as a natural deformity, are very remote.

RESULTS

Distribution of Returns

In the light of all the information collected on faulty marking, regeneration and natural deformities, each scar has been rigidly scrutinized. Table I presents a summary of the distribution of the recoveries from the various experiments.

TABLE I. Summary of recoveries from pink salmon marking experiments in British Columbia.

Year of marking	1931	1932	1932	1933	1935	1936
Area of marking	McClinton creek	Cultus lake	McClinton creek	McClinton creek	McClinton creek	McClinton creek
Source of eggs	McClinton creek	Sweltzer creek	Tlell river	McClinton creek	McClinton creek	Tlell river
Number of migrants	5,400,000 (approx.)		877,648	2,260,000 (approx.)	12,600,000 (approx.)	505,857
Number marked	185,557	8,741	124,002	107,949	85,634	108,200
Mark used	Adipose	Both ventrals	Adipose and left ventral	Both ventrals	Adipose and both ventrals	Adipose and right ventral
Number returned	184	64	40	3,276	35	4
Percentage return	0.1	0.7	0.03	3.0	0.04	0.004
Area of return						
McClinton creek	96			2,941	35	4
Masset inlet (fishing areas)	22			324		
Naden harbour	14			1		
West coast Queen Charlotte islands	2			3		
Karluk, Alaska	8					
South-eastern Alaska	11					
Nass river	1					
Wales island	1					
Chatham sound	1					
Ogden channel				1		
Skeena river	4					
Whale channel				4		
Bear river, Johnstone st.		1		1		
Johnstone strait	7	1				
Deep Water bay or Loughborough inlet	10					
Quathiaski	7					
Deep bay		1		1		
Victoria (Sooke traps)		4				
Fraser river		5	6			
Indian river, Burrard inlet		5				
Gulf of Georgia		22	34			
Cape Flattery		3				
Salmon Banks, Puget sd.		21				
Point Roberts		1				

McCLINTON CREEK—NATURAL RUNS.

1931 test. In 1931, 185,557 fry resulting from the natural spawning of 1930 were marked at McClinton creek by the removal of the adipose fin only. From this there were apparently 184 returns in the autumn of 1932 distributed as follows: McClinton creek—96, Masset inlet fishing areas—22, Naden harbour—14, west coast of Queen Charlotte islands—2, Alaska—19, north coast off Skeena and Nass rivers—7, and Johnstone strait area—24. Superficially such a distribution would appear to demonstrate that while over half of the marked fish returned to McClinton creek or Masset inlet, nevertheless a large number "wandered" to other districts,

as indicated by the recoveries in the outside areas. Such an interpretation must be modified, however, in view of the data presented above on the occurrence of natural deformities in single fins.

In regard to the returns from southeastern Alaska, Dr. F. A. Davidson (Davidson *loc. cit.*) in the spring of 1931 marked 50,000 pink salmon fry at Snake creek, Olive cove, Alaska, by removing the adipose and dorsal fins. The return of eleven adipose fin scars from that area must therefore be treated with caution, since they may be the result of natural deformity or of faulty marking and regeneration in the American experiment.

With the knowledge of proven cases of the appearance of pink salmon in some areas lacking the adipose fins as natural deformities, it would be unsound to conclude that the recoveries made in the 1931 test definitely demonstrated "wandering". Due to the fact, however, that fish lacking adipose fins occurred at McClinton creek in a much greater proportion in the autumn of 1932 than had been indicated by the figures from examinations of other runs, it may safely be assumed that some artificially marked fish returned. Other returns should not be ignored since, until definite information is forthcoming, they may be taken to indicate "wandering" just as much as "homing" is shown by those at McClinton creek.

1933 test. For several reasons the marking at McClinton creek in the spring of 1933 was the most satisfactory of all the experiments and the most productive of reliable results. In the first place, due to the relatively small fry migration it was possible to mark a comparatively large proportion of the total. Secondly, as a result of the experience gained from the 1931 programme, two fins were removed and thus the chance of natural duplication of the mark was practically eliminated. Finally, in 1934 when the recoveries were expected, it was possible to initiate in areas outside of Masset inlet the most efficient system of collection so far employed. An inspector was placed in a representative cannery in each district, and his sole duty was to watch the catches of that plant for pink salmon with missing fins. These canneries included Arrandale on the Nass river, Sunnyside on the Skeena river, Butedale and Namu in the central district, Sooke near Victoria, and Imperial cannery on the Fraser river. One examiner was located in each of two canneries in Masset inlet, several visits were made to the spawning grounds in the tributaries to the inlet in a search for marked fish, and at McClinton creek the usual thorough examination of each adult pink salmon appearing at the weir was carried out. As a result the total recovery was the largest obtained from any pink salmon marking programme up to the present time. For this reason the returns are analysed in more detail than those of the other experiments.

In the spring of 1933, 107,949 pink salmon fry, the progeny of the seeding in McClinton creek the previous autumn, were marked by removing both ventral fins. In the autumn of 1934, 3,276 recoveries were made. Their distribution was as follows: McClinton creek—2,941, Masset inlet fishing areas—324, Naden harbour —1, west coast of the Queen Charlotte islands—3, Ogden channel—1, Whale channel—4, Bear river, Johnstone strait—1, and Deep bay—1. The location of the various areas is shown in figure 4.

The recovery in McClinton creek, the parent stream, of 2,941, or 90 per cent of the total, indicates a strong "homing" tendency on the part of the pink salmon in the year 1934. In addition 324 lacking both ventral fins were captured in the fishing areas in Masset inlet. There is no proof that these fish would not have eventually reached McClinton. The same contention may perhaps be held for those taken in Naden harbour and at Otard bay on the west coast of the Queen Charlotte islands.

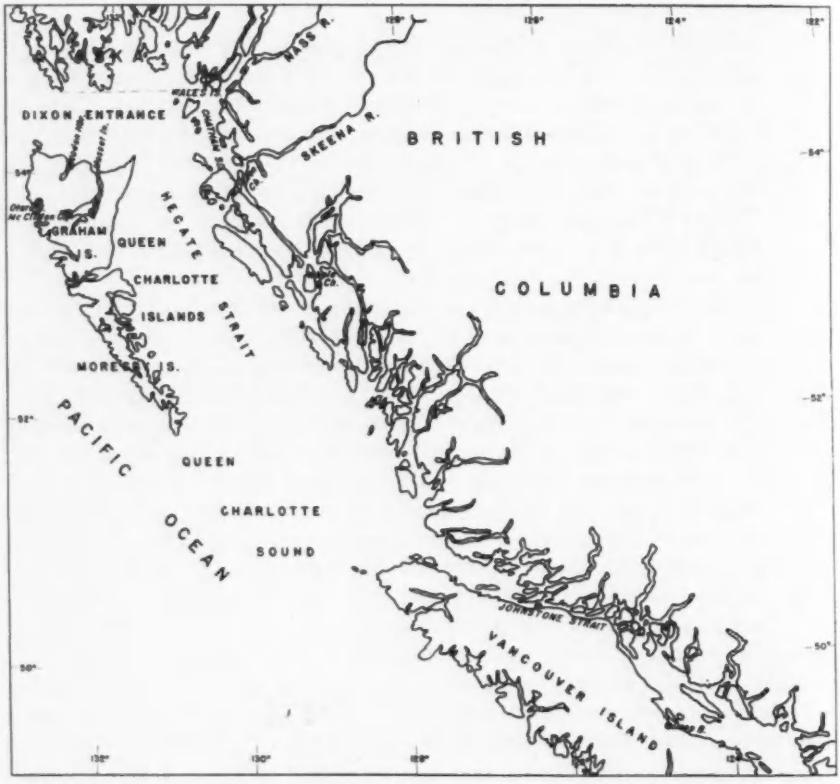

FIGURE 4. Map showing the areas in which recoveries were made from the McClinton creek pink salmon marking experiment of 1933.

There seems very little doubt that the seven remaining returns show that "wandering" may occur. These fish (4 in Whale channel, 1 in Ogden channel, and 1 each from Bear river and Deep bay (Quathiaski cannery)) were taken so late in the season and so far away from McClinton that it is altogether unlikely that they would have been able to return there in time to spawn. Such being the case the presumption of the author on the basis of adipose fin scars in outside areas

(Pritchard 1932) has been justified to some extent. Davidson's theory (Davidson *loc. cit.*), that pink salmon may only stray into more or less neighbouring streams, will have to be extended because in this case the Quathiaski recoveries were almost four hundred miles (645 km.) from the point of marking. It should be stressed, however, that these findings, although they demonstrate "wandering", have not indicated that it occurs to an amount of practical significance.

A detailed examination of the daily returns of fish to McClinton creek during the autumn of 1934 has shown that the marked individuals are evenly distributed throughout the run. The correlation coefficient between the daily count of unmarked fish and that of marked is 0.99 ± 0.0015 which is almost perfect and, of course, highly significant. This discovery is interesting when it is recalled that in the spring of 1933 at the time of actual marking of the fry, half of the total was handled during the first and last portions of the run. While the migration was at its height most of the time was occupied with counting alone. In the ocean phase of the life history these fry must have mixed thoroughly with the general run to produce the even distribution in the returning adults.

1935 test. In the spring of 1935 only 85,634 pink salmon fry were marked by the removal of the adipose and both ventral fins, a number almost negligible in comparison with the total migration of over 12,500,000. In the autumn of 1936 it was found impossible, due to the economic stringency at the time, to put observers in the canneries as in 1934. Although as usual each individual salmon was examined at McClinton creek, reliance again had to be placed on the cannery operators for the returns from outside areas. As intimated in the section on methods, this plan is not efficient since many fish lacking fins are undoubtedly missed in the rush of the busy season.

Under such conditions it was to be expected that the recoveries would be relatively few and preponderantly from the areas where the closest inspection was carried out. Thirty-five individuals were located in the run at McClinton creek and none was reported from any other locality. These data, while they indicate a certain degree of "homing", cannot be accepted as showing that the tendency is perfect since, as above indicated, the lack of recovery of marked fish in outside areas or in the fishery in Masset inlet does not necessarily indicate their absence.

McClinton Fish in Masset Inlet Fishery

With regard particularly to the commercial fishery of Masset inlet, of which McClinton creek is a tributary, the distribution of pink salmon of McClinton creek origin has been indicated by the recoveries of marked individuals. The inlet shown on a very small scale in figure 4 is a body of water about eighteen miles long and six miles wide flowing into Dixon entrance through a narrow channel, one-half to three-quarters of a mile in width and about thirty miles in length (1 mile equals 1.6 km.). Commercial fishing is to a large extent localized off the mouth of the Yakoun river in the southeastern corner and in the various bays emptying into the main body of the inlet. A few hauls are made in the channel which drains into the ocean.

The major fishery is carried out off the mouth of the Yakoun river and it has usually been assumed that the pink salmon taken here would eventually reach the spawning grounds in that stream. The recovery of many marked McClinton creek fish in that fishery demonstrates definitely that more than one stream contributes. The same situation may be said to hold for the area near Buckley bay, off the mouth of the Ain river on the northern side of the inlet. Thus the maintenance of the major concentrations of fish does not only involve the protection of such rivers as the Yakoun and the Ain near which the fisheries are located, but also the guarding of the supply of the other rivers such as McClinton.

TLELL RIVER-McCLINTON CREEK TRANSPLANTATIONS.

A complete discussion of the marking experiments carried out on fry resulting from the Tlell river-McClinton creek egg transplantations has been published (Pritchard 1938).

SWELTZER CREEK-CULTUS LAKE EXPERIMENT, 1932

This is the only marking experiment which has thus far been carried out on pink salmon in southern British Columbia. In the autumn of 1931, Dr. Foerster, who was in charge of the sockeye salmon investigations at Cultus lake, a tributary of the lower Fraser river, took the opportunity to collect some eggs from the pink salmon in Sweltzer creek, the outlet of the lake. These were hatched and the resulting fry retained in ponds until late in the summer of the ensuing year. At that time 8,741 fingerlings were marked by the removal of both ventral fins and released in Sweltzer creek below the fry-counting fence at the lake outlet.

In the autumn of 1933 pink salmon adults lacking the above-mentioned fins were recovered as follows: Off Bear river, Johnstone strait—1; Johnstone strait—1; Deep bay—1; off Indian river, Burrard inlet—5; gulf of Georgia—22; Fraser river—5; Sooke traps—4; cape Flattery—3; Salmon banks, Puget sound—21; and Point Roberts—1.

No pink salmon lacking both ventral fins were taken in the weirs in Sweltzer creek where the young were released nor were any reported from other rivers in the system. The lack of return from different rivers may not be surprising in view of the lack of adequate inspection. The failure of the marked pinks to appear at Sweltzer creek is treated in the general discussion to follow.

Age of Pink Salmon at Maturity

All recoveries from any given marking experiment in British Columbia were received in the autumn of the year immediately after that in which the fry were marked and at no other time. The individuals when recaptured were almost mature and on their way to spawn. Thus the conclusion is justified that pink salmon mature and return to spawn in the autumn of their second year.

The main features of the life history of the pink salmon, viz.—that they leave the rivers as fry almost as soon as they are able to swim and return to spawn in the autumn of their second year, were suggested by Gilbert (1913) from a study of the

scales. In accordance with the habit of migrating as fry the normal scale shows a nuclear area of the sea type with the rings or circuli widely spaced. This area is followed by one in which the rings are closer together representing the slowing of growth in the first winter and finally another area similar to the first in which the rings are again widely spaced, indicating the fast growth of the second summer.

Since the scale reading method is dependent upon the fact that environmental conditions may either speed up or slow down the growth rate and that these changes are registered on the scale in areas in which the circuli are widely spaced or close together respectively, it is to be expected, especially in a fish which reaches such a size as the pink salmon in two years, that other changes in environment than winter or summer will cause "incidental" markings. Davidson (*loc. cit.*, fig. 10) has illustrated a scale from a marked pink salmon known to be two years old in which there appears an additional band of closely spaced rings just inside the annulus which would be normally recognized as the first winter check. This has been interpreted as the effect of unfavourable environment for a short period which has caused a slowing of growth. Similar abnormalities in scale structure have been discovered amongst the marked fish in the British Columbia experiments as well as other types in which the incidental annulus of close rings was situated nearer to the nucleus, in which case it is assumed that the unfavourable conditions acted earlier in the life history.

Gilbert (*loc. cit.*) has stated: "an inner nuclear core of narrowed rings is not infrequently present, and may here also simulate a close-ringed small nucleus of the stream type. . . ." Similar aberrations have been encountered in the scales of marked pink salmon in the present experiments. In these scales the typical markings are also visible. The Cultus lake pinks which were held in fresh water for some time show fairly consistently a core very similar in structure. It is thus justifiable to assume that this check may represent not the end of one year's growth but the effect of a short residence in fresh water. Such residence is possible especially in those larger river systems where it is necessary for the fry to migrate long distances from the upper reaches before entering the sea.

GENERAL DISCUSSION

In all three experiments on the natural runs of McClinton creek, Masset inlet, numbers did return to the "home" stream. Little reliance can be placed on the recoveries from the 1931 and 1935 tests, however, in so far as "wandering" is concerned, due to experimental difficulties and limited collection facilities. For the recoveries of 1934 from the test of 1933, the collection was more efficient and fairly reliable. That year the great majority (90 per cent) returned to McClinton. Only seven (0.2 per cent) were found in areas so far removed from the original one and at a date so late in the season that it could be assumed that they would not come back to the "home" area. It is freely admitted that some might have escaped notice in these outside areas, but in view of the fact that examination was carried on in a large proportion of the canneries, the numbers neglected will be very small in comparison with the total return. The conclusion on the present evidence must there-

fore be that some of the pink salmon in the natural run may wander from the parent stream but the numbers so doing are not economically significant.

Davidson (*loc. cit.*) observes that pink salmon may stray into more or less neighbouring streams. Our findings on the natural runs at McClinton creek indicate that this concession should be extended since one recovery was made at Deep bay, a locality almost four hundred miles (645 km.) distant.

For those fish which resulted from hatchery procedure in the Tlell-McClinton transplantation experiments and the Sweltzer creek test contradictory evidence only is available in this connection. In the case of the Tlell-McClinton transplants the adults in one year appeared at the "adopted" stream, McClinton creek, but in another year they were taken in the Fraser river many miles distant. The fish marked at Cultus lake from the Sweltzer creek eggs appeared in the fishery in southern British Columbia but apparently did not reach the home stream. Whether this difference in behaviour compared to the fish in the natural runs can be attributed to the handling and retention incident to the hatchery procedure cannot be determined on the basis of information at present at hand.

For all the marking experiments on pink salmon it can be stated that in the case of natural runs the majority of fish returned to the parent stream. Wandering in isolated instances may occur in some cases even to approximately four hundred miles from the original area. For those fish which have been raised in hatcheries or have been used in transplantation experiments there appears to be no consistent behaviour in this respect. Further investigation may cause changes in the existing ideas as to wandering and may reveal some reason for the behaviour of artificially raised fish.

Nothing is at present known of the factors involved in causing the salmon to return preponderately to their parent stream. Whether the reaction is the result of purely physical or chemical stimuli in the environment or of some internal reflex in the fish could not possibly be determined from the very small amount of data available. For this reason it is better to discard the word "instinct" in common use at present in referring to this phenomenon, and substitute the more or less non-committal one, "tendency".

SUMMARY

The marking of pink salmon fry as a means of identification of the fish as adults has been employed in British Columbia to determine the age at which the species matures and whether the individuals return to spawn in the same stream in which they hatched as young. The removal of one or more fins involved in this procedure does not appear to harm the fish for at least three or four months after the actual manipulation, but the disproportion of returns in unmarked and marked adults at the parent river suggests that it may handicap the fish during its ocean life. The procedure does not affect the growth or the feeding reactions of the fish. Difficulty is experienced in clipping the fins from small fry cleanly. The remnants of fins will apparently regenerate, and there is evidence to show that new ones may grow for those supposedly removed altogether.

Three experiments were conducted on the natural run at McClinton creek, Masset inlet, B.C. The first of these was faulty in that it involved the removal of the adipose fin only in which natural deformities may occur. In the last the collection system, limited as it was by economic considerations, was unreliable. The other trial showed that the majority of the fish return to the parent stream but that isolated individuals may wander almost four hundred miles from the original area.

In the case of fry used in transplantation or raised in a hatchery and retained for some time there appears to be little consistency in the behaviour in so far as return to a given stream is concerned.

All pink salmon in the McClinton creek run, the transplants from the Tlell river to McClinton creek, and those used from Sweltzer creek, mature and return to spawn in the autumn of their second year. This confirms the original pronouncement of age made from a study of the scales. Incidental checks may occur at least at two places on the scales, as an inner close-ringed central core or as a broad band of close rings in the first widely-spaced area, but these must not be considered as representing the end of a year's growth.

There is no information available as to the factors involved in causing the pink salmon to return preponderately to the home stream.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the facilities placed at our disposal and the active co-operation of the members of the fishing industry and the Dominion Department of Fisheries in the collection of the recoveries from these experiments as well as the efficient services of the examiners. Especial thanks are due Dr. R. E. Foerster for the help in examining all scars and tabulating the data.

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Studies of Fish Spoilage

IV. The Bacterial Reduction of Trimethylamine Oxide

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ABSTRACT

In fish muscle press juice simulating the surface and the interior of muscle, there is an aerobic environment in the surface layer and an anaerobic environment in the body of the liquid. The Eh potential of the former is about 0.3 volts and of the latter from −0.5 to −0.10 volt.

It is found that the bacterial population proliferating at 2°C. is chiefly *Achromobacter*, which can be divided into two groups, obligate aerobes and facultative anaerobes. Only the latter group, which is capable of growth in the interior or surface, is responsible for the reduction of trimethylamine oxide with the evolution of trimethylamine. Since the initial total count is made up of a large number of obligate aerobes or non-oxide reducers it is obvious that the total bacterial population cannot be related to trimethylamine production. The appearance of this base therefore may be taken to indicate a bacterial population which is in excess of that responsible for its production.

Molecular oxygen at surface exercises a trimethylamine oxide sparing effect. In practice, however, this effect is not significant from the point of view of the freshness test in the sense of Beatty and Gibbons.

The importance of trimethylamine estimation as an effective method for determination of the freshness of fish has been demonstrated by Beatty and Gibbons (1937). They found that bacterial increase coincided with the increase in total volatile base and from this deduced that the trimethylamine contribution to the total was directly proportional to the increase in bacteria, as a result of reduction of trimethylamine oxide.

Labrie and Gibbons (1937) advanced a theory accounting for the increase in trimethylamine by a reduction of trimethylamine oxide, initiated by a reduction potential set up by the bacteria when their numbers have reached between 10 and 20 million per ml.

Beatty (1938) has definitely established the precursor of trimethylamine, reporting that even in advanced spoilage of cod muscle press juice it is derived entirely from trimethylamine oxide. The oxide has been found in all fish examined that live constantly in sea water, codfish muscle averaging 0.67 per cent (Beatty 1939).

It having been shown that reduction of trimethylamine oxide is a result of bacterial action, study of the subject at the Atlantic Fisheries Experimental Station has been continued with the purpose of investigating the role of bacteria in the reduction of this compound.

The investigation has included:

(1) A consideration of bacterial environments simulating the surface and interior of fish muscle.

(2) The development of a technique for separation of a group or groups of bacteria responsible for reduction of trimethylamine oxide.

(3) The determination of a relationship between reduction of the oxide and increase in the bacterial population of these groups.

(4) A study of the role of trimethylamine oxide in the metabolism of the bacteria concerned with the reduction.

The first three points of the programme are dealt with in the present paper; the fourth is presented in a following paper.

BACTERIAL ENVIRONMENTS IN FISH MUSCLE

Brooks (1929, 1936) has pointed out that in untreated meats and bacon the muscles after rigor retain indefinitely some of their respiratory activity; thus when they are exposed to air, dissolved oxygen is present only in a superficial layer a few millimetres thick; below this layer the tissue is completely devoid of oxygen. It is logical to assume that fish muscle behaves similarly and that in the centre or directly below the surface there exists an anaerobic state in the bacteriological sense.

To test the validity of the above assumption a method has been employed which depends upon the reversible oxidizable substances and the residual respiratory systems present in such complex substrates as muscle. Because of the influence of oxygen on these systems the *reduction potential* or Eh in the sense of Hewitt (1936) serves as an index to the degree of aerobiosis and anaerobiosis. Thus Brooks (1938) has shown that in the absence of molecular oxygen the reduction potential of muscle tends to drop to a potential of Eh -2.0. Hewitt (1936) reported the attainment of such low potentials in ground tissue.

As pointed out by Labrie and Gibbons (1937) muscle is not suitable for accurate bacteriological and chemical analysis. In the present investigation muscle press juice has been employed throughout. It must be understood that conditions obtained by employing the muscle juice are not entirely comparable to whole muscle. It is suggested, however, that in the following arrangements employing muscle press juice, conditions were sufficiently comparable to muscle for the results to be significant when deducing the probable course of events during fish spoilage. The juice was obtained by pressing ground pre-rigor cod-fish muscle, the temperature being kept at about 5°C. One litre of juice was collected within an hour.

Conditions simulating the surface of fish muscle were secured by placing 25 ml. of press juice in a 500 ml. Erlenmeyer flask. The arrangement is illustrated in figure 1. The flask has three openings in the side; in two of these openings were

placed platinum electrodes; the third opening holds the agar-KCl bridge. The platinum electrodes were made of platinum foil 1 cm^2. These electrodes were carefully prepared before each run, by a cleaning in chromic acid solution and a final electrolysis in a sulphuric acid solution. The electrodes were checked in a standard solution of ferric and stannous chlorides. The platinum foil was placed about 2 mm. below the surface of the muscle press juice. Contact between the calomel cell and the agar bridge was made by dipping the calomel cell into small receptacles containing KCl, fastened permanently to the agar bridges.

Conditions simulating the interior of the muscle were obtained by completely filling stoppered 50 ml. flasks as shown in figure 1. One platinum electrode was placed in each flask, and the agar bridge was similar to the arrangement described above. Each run consisted of four such flasks.

The *potentials* of the surface and of the interior were determined, using a

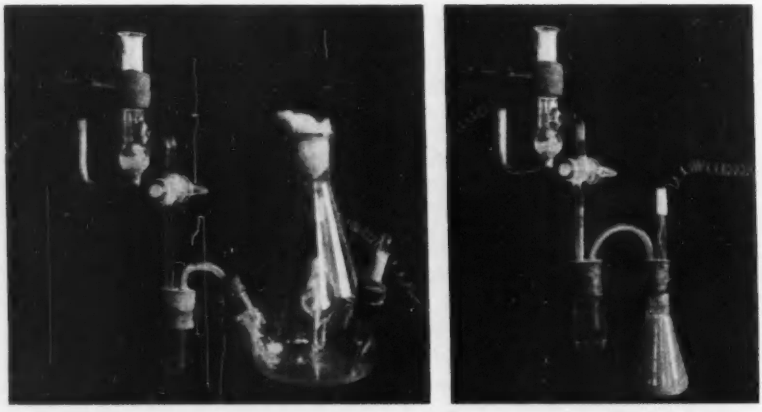

FIGURE 1. Apparatus for the measurement of redox potentials in muscle juice under conditions simulating the surface and interior of muscle.

vacuum tube potentiometer, which enabled readings to be taken without the occurrence of polarization. The flasks were incubated, and the determinations made, at 2°C.

The potential time curves obtained from measurement at the surface and in the interior of the muscle juice are shown in figure 2. The potentials recorded at four electrodes at the surface and three electrodes in the interior are plotted to show the agreement among the electrodes. Only the first 48 hours as recorded in the graphs are now under consideration.

It is evident that muscle juice is heavily poised in the presence of molecular oxygen; in other words, muscle press juice contains reversible oxidizable substances, which when oxidized by molecular oxygen exert a reduction potential in the order of Eh 0.3 volt. If the supply of molecular oxygen is cut off, the potential falls off rapidly to a negative value. It is obvious that the residual respiratory mechanism of Brooks (1936) was exerting an influence on the reduction

potential through oxygen uptake and subsequent reduction of these reversible systems. From the variation between electrodes for the first 24 to 48 hours, it is evident that the system becomes weakly poised in the absence of oxygen; as the reduction proceeds, the system poises itself in the order of Eh −0.08 volt after 48 hours of incubation. This is nearly comparable to that recorded by Brooks (1938) in the interior of meats. This rapid drop in potential does not coincide with bacterial activity, and it may be concluded that fish muscle is comparable to other tissues in its reducing characteristics. Hewitt (1936) has demonstrated

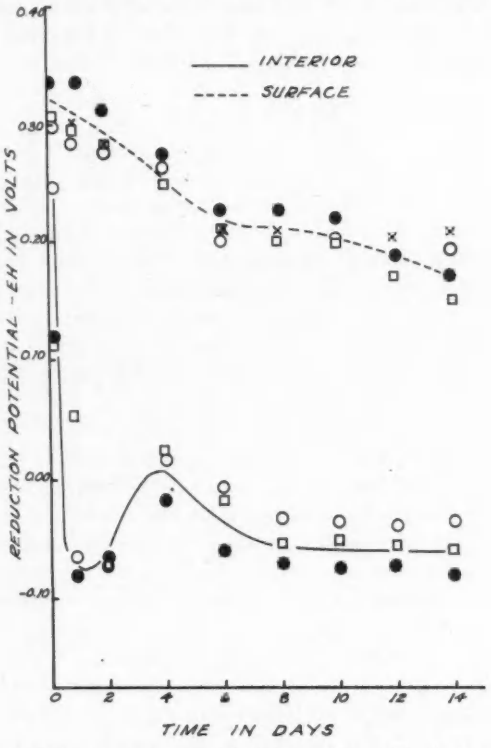

FIGURE 2. Time: potential curves as measured at the surface and in the interior of muscle press juice at 2°C.

a similar effect in serum and attributes the poising to the influence of the oxygen carrying effect of sulphydryl groups present in proteins, auto-oxidizable lipins and haematin compounds.

It may properly be inferred from these results that fish muscle—from a bacteriological point of view—presents two bacterial environments, namely, aerobic and anaerobic. Throughout this investigation, therefore, an attempt has been made to maintain conditions simulating both the surface and the interior of fish muscle, employing muscle juice in the manner described in the present experiment.

SEPARATION OF BACTERIAL GROUPS

It will be shown later that the total bacterial population in the thin layers is greatly in excess of that of the deep layers. If, as was assumed by Beatty and Gibbons (1937), the reduction of trimethylamine oxide is proportional to the total number of organisms, the amount reduced in the shallow layer should be greatly in excess. But since the rate of reduction is approximately the same, it follows that it depends not on the total population but on the types of bacteria present. Thus, before it was possible to correlate bacterial growth with trimethylamine oxide reduction it was necessary to develop a method for the determination of the group or groups of bacteria responsible for the reduction.

Principle and Method of Separation

It was found that certain bacteria growing in muscle press juice can grow anaerobically in the presence of trimethylamine oxide and an oxidizable substrate. All organisms growing on the anaerobic plates were tested aerobically and grew well, that is, they were able to utilize both trimethylamine oxide and oxygen as hydrogen acceptors. Using Spray's (1936) anaerobic plating medium it was not possible to demonstrate obligate anaerobic bacteria, although Shewan (1938) isolated a few species from haddock feces. Hence anaerobic counts, which are described later, represented the facultative anaerobes, and the difference between aerobic counts and anaerobic counts, the obligate aerobes.

Preparation of Media and Counting Technique

With the reduction of the oxide there is a liberation of the base, trimethylamine. Thus, in unbuffered media containing trimethylamine oxide, a slight reduction of the oxide may immediately place the reaction of the medium out of the physiological range. This point is clearly demonstrated in figure 3, in which it will be seen that beef extract broth, being poorly buffered, undergoes a rapid shift in reaction on the addition of even small amounts of alkali. On the other hand, cod muscle press juice is highly buffered, as will be observed. As an index to the degree of buffering, the media employed have been buffered to the same extent as cod muscle press juice by the addition of phosphates; a comparison between the buffering capacity of press juice and the buffered medium is shown in figure 3.

Throughout the investigation strict control of hydrogen-ion concentration and of buffering capacity of the medium was maintained; the determinations were made with the aid of a glass electrode. The medium—the buffering curve of which is shown in figure 3—was prepared as follows: Beef extract 3.0 g., peptone 5.0 g., sodium chloride 5.0 g., potassium phosphate (KH_2PO_4) 6.8 g., sodium phosphate ($Na_2HPO_4.12H_2O$) 18.0 g., agar 15.0 g., dissolved in water, adjusted to pH 6.9 with sodium hydroxide, and made up to 1 litre.

This medium was used for all the aerobic counts; the anaerobic counts were made on the same medium plus 7.5 g. trimethylamine oxide per litre, which is a concentration of the order found in cod muscle press juice. The medium was placed in test tubes which contained approximately 11 ml. During the auto-

claving there was a slight precipitate of insoluble phosphate, but by using individual tubes each plate contained the same amount of precipitate as well as the same volume of medium. The precipitate was crystalline and easily distinguishable from bacterial colonies when magnified 14 diam.

The dilution fluid was also buffered and prepared as follows: Sodium chloride 10.0 g., potassium phosphate (KH_2PO_4) 4.5 g., sodium phosphate ($Na_2HPO_4.12H_2O$) 12.0 g., dissolved in water, adjusted to pH 6.9 with sodium hydroxide, and made up to 1 litre.

The counting technique was based on that of Jennison (1937). Duplicate dilutions and triplicate plates from each dilution were employed for each count; nine ml. of the above dilution fluid were placed in individual test tubes; sterilized

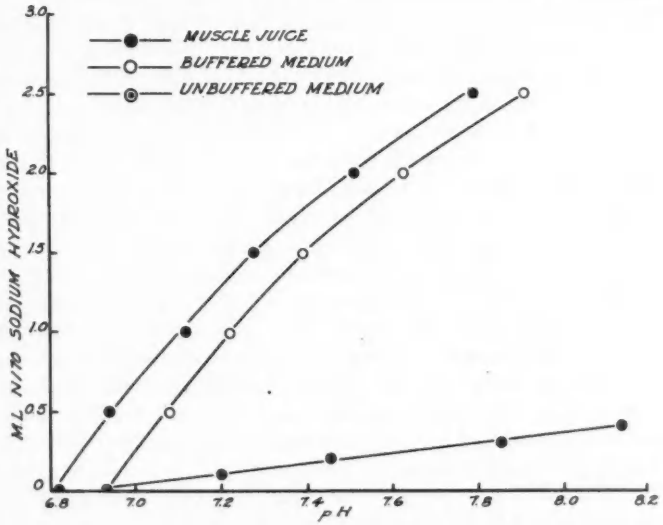

FIGURE 3. The buffering capacity of unbuffered and buffered media as compared to muscle press juice.

corks were used to plug the tubes during mixing. The smaller volumes require a greater number of dilution blanks for each count but assure an even distribution of organisms. Counting conditions were maintained as uniformly as possible.

All plates were incubated at 25°C. and counted after four days. The problem presented by the necessity of making anaerobic counts in large numbers was solved by the designing of an economical and controlled anaerobic chamber which is described in a subsequent paper.

BACTERIAL RELATIONSHIP TO REDUCTION OF TRIMETHYLAMINE OXIDE

METHODS

The reduction of trimethylamine oxide was determined and recorded as the increase in trimethylamine nitrogen. Values above 40 mg. per cent were deter-

mined, employing the Beatty and Gibbons (1937) modification of the Parnas-Mozolowski apparatus. The initial and lower values were determined by the Beatty and Gibbons (1937) adaptation of the Conway and Byrne (1933) method for the measurement of ammonia.

A further modification of the Conway and Byrne method for the determination of trimethylamine has been made, because of the difficulty in foretelling the necessary quantity of standard acid as employed in the Conway apparatus, especially during the logarithmic increase in trimethylamine. This difficulty was largely overcome by substituting boric acid for the standard acid. With this modification it was possible to cover a range from 0.0 to 40.0 mg. per cent nitrogen employing two boric acid solutions. The method had a further advantage in that the boric acid could be measured with a pipette; the accuracy of this measurement was not important. The required strengths of boric acid solutions were determined by means of titration curves; it was found that trimethylamine was trapped until the hydrogen-ion concentration approached pH 8.0. From these curves it was possible to determine the minimum strength of boric acid necessary to trap a definite quantity of trimethylamine. Moreover it was necessary to keep the concentration of boric acid at a minimum since the buffering tended to decrease the sensitivity of the indicator. In measuring from 0 to 10 mg. per cent nitrogen 1 ml. of 0.1 M. solution of boric acid was used, and from 10 to 40 mg. per cent nitrogen 1 ml. of 0.3 M. solution. The solutions were made up in 250 ml. quantities; in making up this amount 10 ml. of Conway and Byrne's (1933) modification of Tashiro's methyl red methylene blue indicator was added, plus sufficient alkali to bring the indicator to the neutral point; the solution was finally brought up to volume. It was necessary to prepare fresh boric acid solutions at least every two weeks, since the indicator deteriorated, making the end point difficult to detect. The back titrations were carried out in the usual manner, using microburettes containing standard acid. It was necessary to employ two acids, one for low trimethylamine values and a stronger solution for the high values. The accuracy of the method was tested on pure solutions of trimethylamine; the base could be recovered within 1.0 per cent.

Experimental

Surface conditions were obtained by placing 25 ml. of muscle press juice in each of a number of 500 ml. Erlenmeyer flasks (depth 4 mm.) plugged with cotton in the conventional manner. Conditions simulating the interior were obtained by filling 50 ml. flasks and stoppering tightly. A sufficient number of flasks were prepared so that the whole contents of each flask could be used for bacteriological and chemical analysis; this technique prevented error due to sampling. It was found important to mix the muscle juice thoroughly to assure an even distribution of bacteria. The flasks were incubated at 2°C.; individual flasks were removed at intervals of two days for analysis.

The results are given in figure 4. On examining the curves the outstanding observation is the influence of the environments. The trimethylamine curves show that the availability of molecular oxygen at the surface resulted in a trimethylamine oxide *sparing* effect. In this respect the effect was most evident for

the first four days of incubation, after which the reduction of the oxide at the surface was nearly comparable to that of the interior. The phenomenon will be explained later.

On examining the curves of the total aerobic population and comparing them with the trimethylamine curves, it will be noticed that the number of bacteria decreased for the first two days, while the trimethylamine increased over the same period; this was not always true as shown from repeated experiments, but in no case was there a relationship between the total count and trimethylamine increase over this period. From a consideration of the area between these curves it may be concluded that a group of organisms dependent upon molecular oxygen were proliferating at the surface.

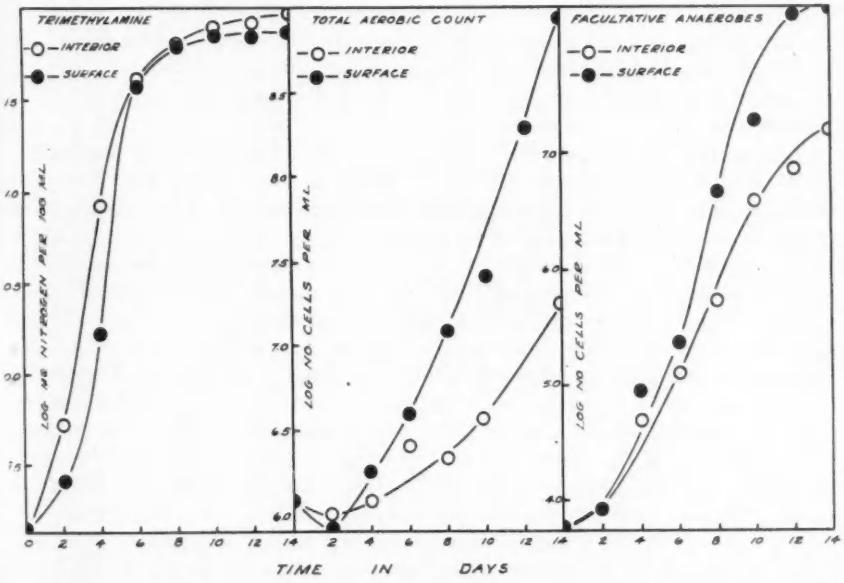

FIGURE 4. Relationship between trimethylamine increase, total aerobic population and facultative anaerobes as determined at the surface and interior of cod muscle press juice at 2°C.

On comparing the facultative anaerobic curves with the trimethylamine curves there is evidence of a close relationship. It is evident that the numbers were greater at the surface since they were able to take advantage of the molecular oxygen present, while those in the interior had to rely upon hydrogen acceptors other than molecular oxygen.

If the data used for the facultative anaerobic curve of the interior are subtracted from the data for the total aerobic count of the interior, the resultant curve will actually show a decrease in number, which indicates that obligate aerobes were unable to grow in this environment. If the same procedure is applied to the surface curves, the resultant curve will show a rapid increase in

numbers, indicating the rapid proliferation of obligate aerobes at the surface. This point is effectively illustrated in figure 5 where the obligate aerobic numbers were obtained and plotted in this manner. These obligate aerobes made up a large portion of the initial total population which therefore cannot be related to the evolution of trimethylamine, as was done by Beatty and Gibbons (1937) and Labrie and Gibbons (1937) before this differentiation was possible.

Influence of Aeration

In repeated experiments it was possible to increase the area between the trimethylamine curves plotted in figure 4, by decreasing the depth of the surface layer. It was assumed that the availability of molecular oxygen was increased, exerting an oxide *sparing effect*.

To magnify this effect and thus confirm the assumption, the above experiment, the results of which are plotted in figure 4, was repeated, determining the influence of strong aeration on the reduction of trimethylamine oxide. To increase the aeration, a stream of air with sufficient force to keep the thin layer of muscle juice in continual movement was passed over the surface.

Facultative anaerobic and obligate aerobic bacterial counts were determined as described in the previous experiments. In view of the hypothesis of Labrie and Gibbons (1937) oxidation-reduction potentials were measured in the same manner as described in the first section of this paper.

The results of the first six days of the experiment have been plotted in figure 5, since the oxide *sparing effect* was most evident in this period. The "aerated data" and "non-aerated data" are from the results of two different experiments, using different muscle juice; they were controlled by running parallel experiments under conditions imitating the interior of muscle. The results confirm the previous assumption, aeration increasing the area between the trimethylamine curves of the aerated experiment and the control. The extreme aeration prevented the reduction of trimethylamine oxide for the first 48 hours. When the bacteria began to proliferate rapidly, as shown from the facultative anaerobic curves, the oxide was reduced regardless of the aeration. The bacterial growth curves were not influenced significantly for the first six days; on following days, the obligate aerobes at the aerated surface proliferated rapidly as compared to those at the non-aerated surface. The oxidation-reduction potential curves confirm those plotted in figure 2; these curves were not altered significantly by the aeration. As shown in figure 5, the surface electrodes were poised at Eh 0.3 volt; active proliferation produced a drop to the order of Eh 0.2 volt. The potential as recorded at the electrode in the muscle juice simulating the interior shows the same reduced environment as recorded in figure 2. The characteristic trend as recorded at these electrodes between 2 and 6 days has been evident in all experiments; it is probably indicative of the oxidation or reduction of a system having a characteristic potential within this range.

It is evident that fish muscle press juice is heavily poised and therefore the potential as measured at a platinum electrode is no criterion of the potential induced at cell surfaces. Yudkin (1935) came to the same conclusion employing cell suspensions and weakly poised solutions. The fact is that during the loga-

rithmic growth phase, the facultative anaerobic *Achromobacter* were able to set up the required reducing intensities at their cell surfaces for the activation of trimethylamine oxide as hydrogen acceptor, while at the same time, the potentials

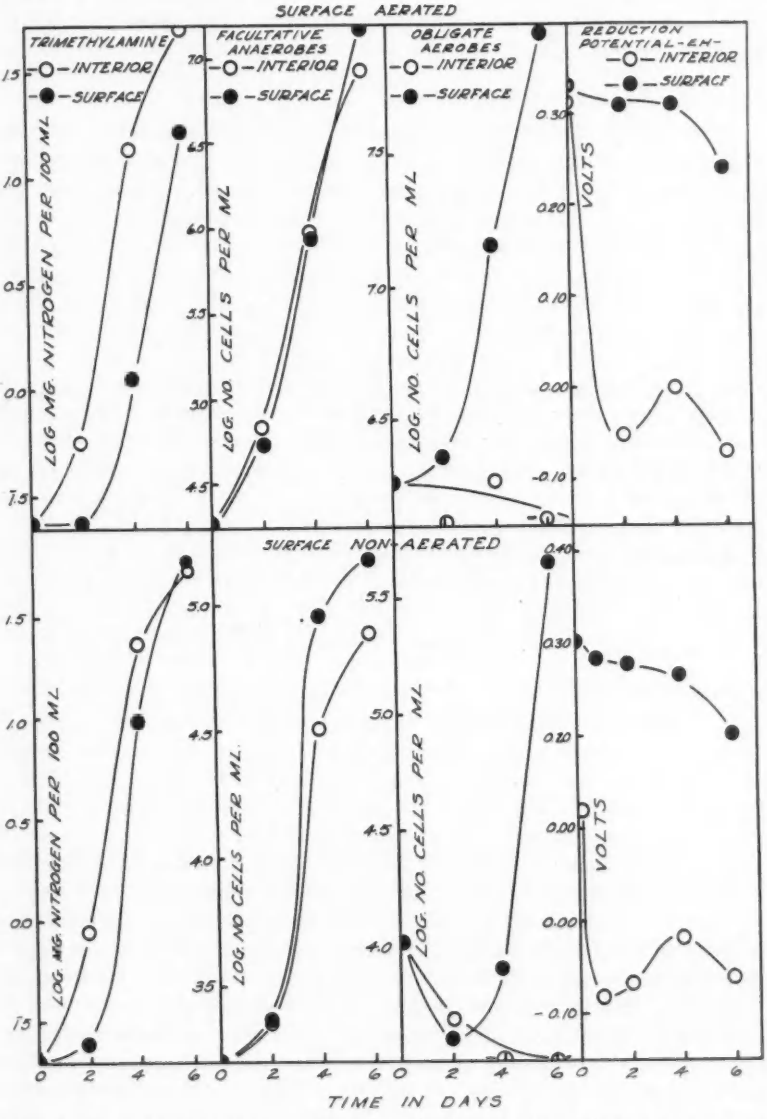

FIGURE 5. Influence of aeration on the trimethylamine increase, obligate aerobes, facultative anaerobes and reduction potentials at the surface of cod muscle juice at 2°C. as controlled by similar measurements in the interior.

recorded at the electrode indicated that their environment was poised at a relatively high potential of Eh 0.2 to 0.3 volt. The hypothesis of Labrie and Gibbons (1937) on the reduction of trimethylamine oxide assuming the attainment of a critical potential of the environment must be referred, not to the conditions of the media, but to the surface of the cell where the trimethylamine oxide has a metabolic significance.

Discussion of "Sparing Effect"

Stephenson (1930) has pointed out that facultative anaerobes can effect their oxidations more profitably from molecular oxygen than through anaerobic oxidations by hydrogen acceptors. This was observed when the reduction of trimethylamine oxide was prevented by intense aeration during the first 48 hours when the bacterial increase was not great. When the numbers greatly increased even this failed to stop the reduction of the oxide, for as Rahn (1932) and Anderson (1938) have pointed out, the solubility of oxygen is small and it soon became insufficient to support increasingly large numbers. This led to the utilization of the hydrogen acceptors which were in solution.

This *sparing effect* as applied to fish muscle surfaces would not be significant. As already shown there are many bacteria at the surface which depend entirely on molecular oxygen for their oxidations, setting up a competition for the dissolved oxygen present. The aeration necessary to make the phenomenon significant would never occur at muscle surfaces; and would have no attenuating influence on the reduction of trimethylamine oxide. Beatty and Gibbons (1937) have shown that the rate of trimethylamine production was greater at surface tissues such as gills and peritoneum than in muscle tissues. This can be explained by the difference in contamination between the two areas and the inefficiency of the transport of oxygen to the cells as described above.

The Organisms Concerned

No extensive systematic study has been made of the facultative anaerobes or the obligate aerobes except that required to place them in their respective genera. Some two hundred species that reduce trimethylamine oxide were studied. The majority of the species in this group were members of the genus *Achromobacter*, although there were a few species of lactose splitting types. The obligate aerobic types belonged to the *Flavobacter* and *Achromobacter*; after 4 or 5 days of incubation at 2°C., species present in muscle juice other than *Achromobacter* were not significant. It may be concluded, therefore, that the genus *Achromobacter* is chiefly responsible for the spoilage of cod muscle at low temperatures. These results are in agreement with those of other authors. Gibbons(1934) and Stewart (1932) have made systematic surveys of bacteria associated with spoiling fish and found members of the genus *Achromobacter* most abundant.

Throughout the remainder of the investigation the *Achromobacter* have been divided into the groups depending on their ability to reduce trimethylamine oxide,—(*a*) *reducing Achromobacter* and (*b*) *non-reducing Achromobacter*. There is, however, the possibility of either group becoming adapted to the other condition.

As previously stated, obligate anaerobes play no part in the initial stages of fish spoilage.

A few species of microaerophilic, acidogenic strains were isolated; but they were never present after the initial count. Studies revealed that they were unable to reduce trimethylamine oxide and indicated their inability to proliferate at 2°C. They appear to be typical of the strains isolated by Shewan (1936), and the *Lactobacilli* of Watson (1938).

Reduction by Pure Cultures

It is evident (figure 4) that there is a relationship between the growth of mixed cultures of facultative anaerobes and the production of trimethylamine, as is also the lack of such in the growth of mixed culture of obligate aerobes. This was further examined by using pure cultures prepared from the same material.

Separate strains were selected from mixed cultures appearing in the plates used for the 8 and the 12 day counts, in figure 4. These were inoculated on to media containing trimethylamine oxide equivalent to 40 mg. nitrogen per 100 ml. of medium. Those strains capable of anaerobic growth in the previous experiment successfully reduced the oxide, while those not capable of anaerobic growth failed to do so.

Reduction Related to Growth of a Pure Strain

The ordinary stock medium minus the agar with the addition of trimethylamine oxide equivalent to 70 mg. nitrogen per 100 ml. was placed in 10 ml. lots in a series of test tubes. A tube of the above medium was inoculated from a test culture of a typical member of the facultative anaerobe group as isolated above (reducing *Achromobacter*) and incubated for 12 hours at 25°C. This sub-culture was thoroughly mixed and 0.1 ml. was placed in each of the above tubes. After the cultures were mixed, they were incubated at 25°C.; tubes were removed at two hour intervals for analysis; total counts and trimethylamine determinations were made.

The results are expressed graphically in figure 6. It will be observed that the reduction of the oxide closely paralleled the growth of the organism.

Reduction of Trimethylamine Oxide and Nitrates Correlated

Hess (1934) has demonstrated the ability of *Achromobacter* to adapt its metabolism to the reduction of nitrates at low temperatures; in the light of the findings of Quastel, Stephenson, and Whetham (1925) these bacteria were carrying on respiration at these low temperatures employing nitrates as hydrogen acceptors. If this conclusion is correct, additional significance would be added to the trimethylamine test of Beatty and Gibbons (1937) when it is considered that Hess observed this adaptation to the rapid reduction of nitrates at temperatures in the order of 0° to −3°C.

Buffered stock medium, made semi-solid by the addition of 1.5 g. of agar per litre, plus 0.1 per cent potassium nitrate, was used for the nitrate reduction tests. The oxide medium was prepared from the stock medium as described above, but

with the addition of trimethylamine oxide equivalent to 70 mg. per cent nitrogen. Nitrate reduction was determined by a qualitative test for nitrites and the oxide reduction by quantitative determinations for trimethylamine. The organisms were secured from a 14 day count on cod muscle juice formerly incubated at 2°C. The cultures were incubated at 25°C. for 48 hours before testing.

The results are set forth in table I. It is evident that all *Achromobacter* capable of the reduction of trimethylamine oxide also reduced nitrates; the species not reducing nitrates cannot reduce the oxide. It is interesting to note that the *Flavobacter* studied were able to reduce nitrates but not the oxide.

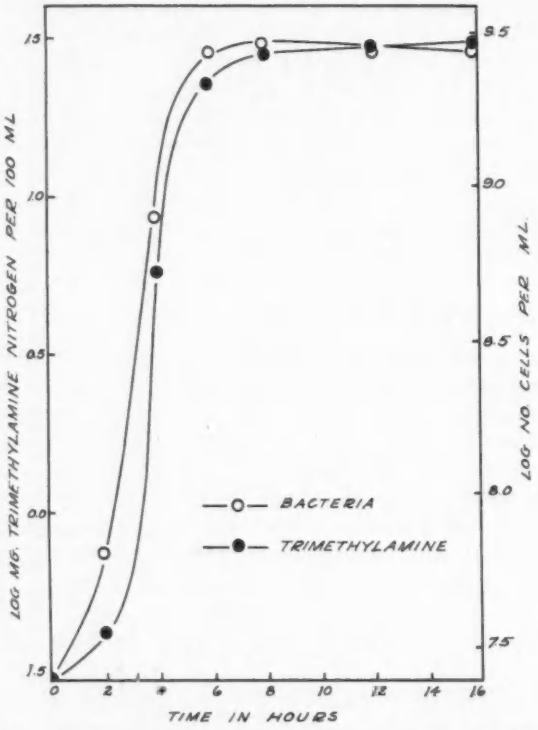

FIGURE 6. The relationship between trimethylamine oxide reduction and the bacterial increase of a pure strain of *reducing Achromobacter* at 25°C.

TABLE I. The relationship between the reduction of trimethylamine oxide and that of nitrates by bacteria isolated from cod muscle press juice.

Bacteria represented	NO_3 reduction (nitrite)	$(CH_3)_3NO$ reduction (mg. nitrogen)
50×10^6 (*non-reducing Achrom.*)....	−	0.48
50×10^6 (*reducing Achrom.*).....	+	68.0
10×10^6 (*Flavobacter*).....	+	0.22
Control.....	−	0.50

SUMMARY

Oxidation-reduction potentials have been determined in cod muscle press juice under conditions simulating the surface and the interior of the muscle. The Eh potential of the surface is in the order of 0.3 volt and that of the interior is −0.05 to 0.10 volt. These results indicate the existence of an aerobic and anaerobic environment in the bacteriological sense.

Boric acid has been substituted for standard acid in the method of Conway and Byrne; this enables a rapid and convenient method for the determination of trimethylamine.

The reduction of trimethylamine oxide during the spoilage of cod muscle press juice at 2°C. is not a function of the total bacterial population. The bacteria are divided into *non-reducing Achromobacter* and *reducing Achromobacter*. The former group are obligate aerobes and proliferate only at the surface in the presence of molecular oxygen; the latter group are facultative anaerobes and grow at the surface and in the anaerobic interior at the expense of hydrogen acceptors, excluding trimethylamine oxide. Obligate anaerobes and species other than *Achromobacter* are not significant in the initial spoilage period.

There is no direct correlation between the reduction potential of the environment external to the cell and the reduction of trimethylamine oxide.

Oxygen present at the surface produces an oxide "sparing effect" which is increased on aeration. The significance of the phenomenon is discussed.

The reduction of nitrate as a hydrogen acceptor by the *reducing Achromobacter* is comparable to the reduction of trimethylamine oxide. The importance of this observation is pointed out.

The reduction of trimethylamine oxide by pure cultures of *reducing Achromobacter* under laboratory conditions is reported. The results show a parallel relationship between the reduction of the oxide and the growth of a pure strain.

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Studies of Fish Spoilage

V. The Role of Trimethylamine Oxide in the Respiration of Achromobacter

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(*Received for publication October 24, 1938*)

ABSTRACT

A general equation involving the reduction of trimethylamine oxide by *Achromobacter* is derived and tested, and is $AH_2+(CH_3)_3NO \rightarrow A+(CH_3)_3N+H_2O$, where AH_2 is the hydrogen donator and A the oxidized substrate. The reduction of trimethylamine oxide as hydrogen acceptor with the evolution of trimethylamine is a linear function of time in the presence of cell suspensions and single hydrogen donators including glucose, glycogen, lactate, and pyruvate. All strains of *Achromobacter* are not able to reduce the oxide, although they may contain the same dehydrogenases as revealed by employing the methylene blue technique. Small concentrations of hydrogen acceptors such as nitrate and methylene blue inhibit the reduction of the oxide. Since fumarate is not inhibitive and supports anaerobic growth there is evidence of a preferential activation of hydrogen acceptors.

The present investigation deals with the last point of the programme outlined in the preceding paper (Watson 1939), namely, the role of trimethylamine oxide in the metabolism of the bacteria responsible for its reduction. As described in that paper, a method was developed for differentiating the bacteria proliferating in cod muscle press juice into *reducing* and *non-reducing Achromobacter*, owing to the ability of the former group to grow anaerobically in the presence of trimethylamine oxide and an oxidizable substrate.

The evidence presented in the foregoing paper suggested trimethylamine oxide functioning as a hydrogen acceptor in the anaerobic growth of the reducing *Achromobacter*. Wieland's theory of hydrogen transfer has been extensively applied to bacterial dehydrogenations by Quastel and Whetham (1924, 1925a, 1925b), Quastel, Stephenson and Whetham (1925) and Quastel and Wooldridge (1925). These authors have shown the importance of methylene blue, fumarate, nitrates, chlorates and many other compounds in the role of hydrogen acceptors during the anaerobic respiration of bacteria. Krebs (1937) has made a thorough study of fumarate as hydrogen carrier in the respiration of *B. coli*. He concluded that fumarate, pyruvate, oxaloacetate and probably carbon dioxide act as respiratory catalysts.

In order to determine the role of trimethylamine oxide in the respiration of *Achromobacter* there were included in this investigation: (1) the measurement of the ability of cell suspensions of both *reducing* and *non-reducing Achromobacter*, as defined in the previous paper, to reduce hydrogen acceptors including trimethylamine oxide in the presence of different hydrogen donators; (2) the determination of the influence of various hydrogen acceptors on the reduction of trimethylamine oxide; (3) a quantitative investigation of the coupled oxidation-reduction reaction between lactic acid as hydrogen donator and trimethylamine oxide as the acceptor in the presence of *reducing Achromobacter*.

THE REDUCTION OF HYDROGEN ACCEPTORS

With Different Substrates

It was planned to investigate the reduction of the oxide employing cell suspensions of the organisms in conjunction with pure substrates for hydrogen donators. The two groups of *Achromobacter* each include many species. In the present study *reducing Achromobacter* and *non-reducing Achromobacter* are used to designate single pure strains typical of these groups; the same strains have been employed throughout the investigation.

The advantages of the cell suspension technique have been adequately outlined by Wilson (1938).

The stock medium plus trimethylamine oxide (Watson 1939) placed in Kolle flasks was employed to grow the organisms. After 24 hours' incubation the growth was washed off with a buffered saline solution made similarly to the dilution fluid as described in the previous paper. The cells were washed and centrifuged twice, the suspension of them brought to a definite volume, and the number of cells per ml. of suspension determined; 1 ml. of suspension contained in the order of 65×10^9 cells. The suspensions were stored at 10°C. and generally used within 24 hours after preparation.

The hydrogen donators used were glycogen, glucose and lactic acid made up in buffered saline solution which was finally adjusted to a hydrogen-ion concentration of pH 6.9. These carbohydrates and carbohydrate derivatives were chosen because Sharp (1934, 1935) demonstrated a continuous breakdown of glycogen and accumulation of lactic acid, and Beatty and Collins (unpub.) showed that lactic acid disappears concomitantly with the production of trimethylamine.

In the present and subsequent experiments strict control of hydrogen-ion concentration was maintained; reaction mixtures, cell suspensions and media were adjusted and buffered to a hydrogen-ion concentration of the order of pH 6.9, as determined by the glass electrode.

To test the reduction of the oxide in the presence of these hydrogen donators, six series, each of approximately ten tubes, were made up. Three ml. of trimethylamine oxide solution were added to each tube, the final concentration being 50 mg. nitrogen per 100 ml. of medium. Glucose solution was added to two series making a final concentration of 0.5 per cent; one series contained 1.0 per cent lactic acid; one series was made up to 0.5 per cent glycogen; the remaining series were controls in that no hydrogen donators were added. Five ml. of bac-

terial suspension (*reducing Achromobacter*) were added to each tube in one glucose, the glycogen, and one control series; a similar quantity of *non-reducing Achromobacter* suspension was added to each tube of the remaining series. These tubes were stoppered tightly and incubated at 30°C. A tube from each series was removed every two hours for analysis. The reduction of the oxide was followed by making trimethylamine determinations by the Conway and Byrne method (Watson 1939).

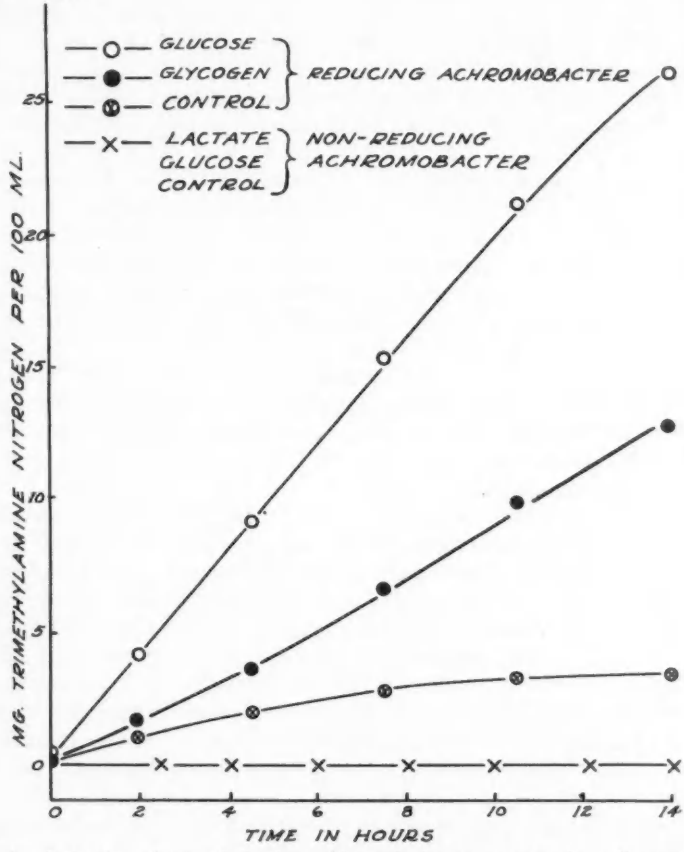

FIGURE 1. Reduction of trimethylamine oxide as hydrogen acceptor by cell suspensions of *Achromobacter* in the presence of different donators.

The results are expressed graphically in figure 1. It will be observed from the curves that the *non-reducing Achromobacter* were unable to reduce the oxide in the presence of the substrates, but the *reducing Achromobacter* did so readily, thus confirming previous observations (Watson 1939). The oxide was reduced as a linear function of time. In the absence of the donator, as shown from the control there was very little reduction of the oxide, that during the first 8 hours

being accounted for by the oxidizable substances present in the suspension. This effect has been reduced in subsequent experiments by aerating the suspension at room temperature and re-centrifuging before making up to volume.

As a Measure of Dehydrogenase Activity

From the results of the above experiment it may be concluded that under the given conditions trimethylamine oxide plays no part in the respiration of *non-reducing Achromobacter*. In view of the possibility of this being due to adaptation (Watson 1939), a comparative study of respiration in these two groups of *Achromobacter* has been undertaken.

The methylene blue technique of Thunberg (Meldrum 1934) has been employed. A modification of the inverted U-tube of Quastel and Whetham (1924) was used as a reaction chamber; this tube will be described later. The bacterial suspension was placed in one arm, and the methylene blue and hydrogen donator in the other. After evacuating the air and refilling with oxygen-free nitrogen, the tube was incubated in a water bath at 30°C. When the temperature became constant the contents of both arms were mixed and the time recorded for complete reduction. The final concentration of methylene blue was 0.001M. and the concentration of each donator was of the order of 0.025M. For the trimethylamine oxide reduction tests, a series of tubes was prepared as described in the preceding experiment. The concentration of the donators was 0.025M. and the final concentration of trimethylamine oxide was equivalent to 8 mg. nitrogen per 100 ml. As in the methylene blue technique, 5 ml. of bacterial suspension were added to each tube. The tubes were incubated at 30°C. and the per cent reduction determined from the trimethylamine values.

The results have been recorded in table I. It is evident that the *reducing Achromobacter* and *non-reducing Achromobacter* contained active dehydrogenases when methylene blue was employed as hydrogen acceptor. On the other hand, trimethylamine oxide was reduced as hydrogen acceptor only by the *reducing Achromobacter*. In considering these results it should be recalled that methylene blue is a reversible system and does not require activation by the cell in the sense of other hydrogen acceptors such as nitrate and fumarate. From these results it is evident that trimethylamine oxide, like nitrate, requires a degree of activation by the cell. Thus, *non-reducing Achromobacter* either have lost the necessary activating power through the influence of their environment or have never possessed it.

Table I. Dehydrogenase activity of *Achromobacter* as measured by the reduction of methylene blue and trimethylamine oxide.

Donator	Methylene blue (min. for 100% reduction)		Trimethylamine oxide (% reduction in 11 hours)	
	Achromobacter		*Achromobacter*	
	reducing	non-reducing	reducing	non-reducing
Glucose	50	80	66.0	0
Glycogen	70	113	26.2	0
Lactic acid	80	30	35.5	0
Pyruvic acid	70	60	53.0	0
Control	∞	∞	2.1	0

AS AN INDEX TO OXIDATION OF HYDROGEN DONATORS

The technique varied from the first experiment in that each series was contained in a 50 ml. Erlenmeyer flask. The samples were pipetted for analysis and anaerobic conditions were maintained by passing oxygen-free nitrogen through the mixtures. The final concentration of donators was 0.05 M., while the tri-

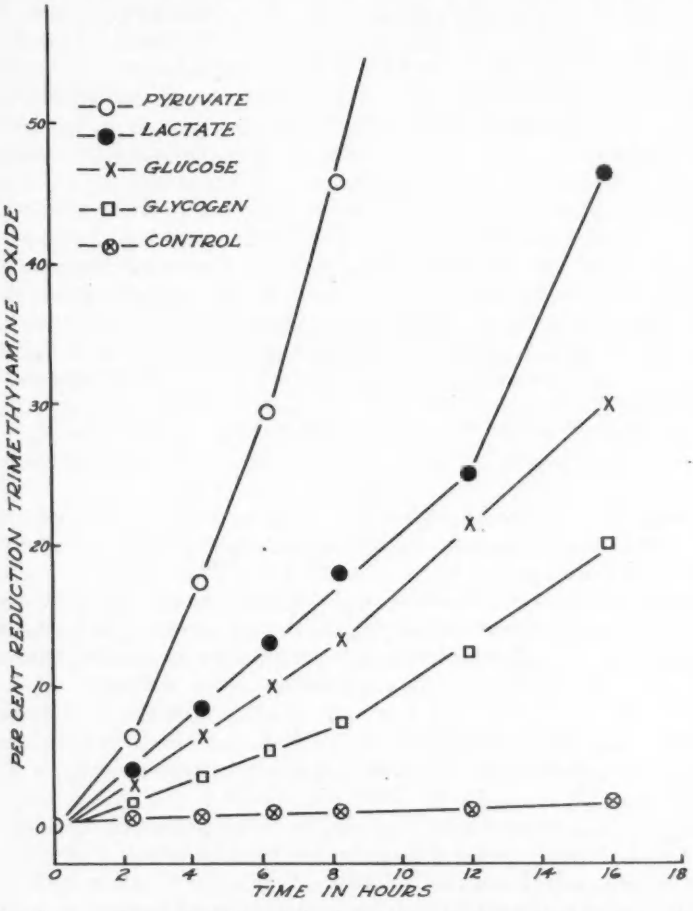

FIGURE 2. Comparative rates of trimethylamine oxide reduction as an index to oxidation of hydrogen donators by a cell suspension of *reducing Achromobacter*.

methylamine oxide content was equivalent to 28.0 mg. nitrogen per 100 ml. One series was maintained as a control in that it contained no hydrogen donator. A bacterial suspension of *reducing Achromobacter* was added to each series so that 1 ml. of the final solution contained 4×10^9 cells. The flasks were incubated in a constant temperature bath at 30°C. The percentage reduction of trimethylamine

oxide was determined by the estimation of trimethylamine at intervals of approximately two hours.

The results are expressed graphically in figure 2. It will be observed that the reduction of the oxide was a linear function of time as seen in figure 1. It is obvious that pyruvate was oxidized more rapidly than lactate. Moreover, from table I it will be noticed that after 11 hours this increase in oxidation of pyruvate was accompanied by an increased reduction of trimethylamine oxide. The relative rates of reduction in the presence of glucose and glycogen and the failure of the oxide to be reduced in the control approximate the results as plotted in figure 1. From the results in figure 2 it will be observed that there are definite changes in rates with time, most evident for the pyruvate curve, and next for the lactate. Krebs (1937) has observed this for pyruvate, employing fumarate as hydrogen acceptor. An explanation has not been advanced.

It is seen in table I that glucose has a higher reducing activity than the other donators, but in figure 2 that both pyruvate and lactate have a higher reducing activity than glucose. The relationship, however, between glucose-glycogen and pyruvate-lactate always remains the same; in other words, glucose displayed more reducing activity than glycogen and pyruvate was oxidized more rapidly than lactate. This discrepancy in dehydrogenase activity may be explained in the light of the adaptive enzyme phenomenon as discussed by Knight (1936) and Anderson (1938).

INTERACTION OF OTHER HYDROGEN ACCEPTORS

The influence of other hydrogen acceptors on the reduction of trimethylamine oxide was determined both by growth experiments and in the presence of cell suspensions.

In the growth experiment four series of tubes were set up each containing 10 ml. of the stock medium of Watson (1939), made semi-solid by the addition of 1.5 g. agar per litre, and with trimethylamine oxide to give a final concentration of 20 mg. nitogen per100 ml. To each of three series methylene blue, nitrate or fumaric acid was added to a final concentration of 0.002 M. and the fourth series kept for control. Individual tubes of each series were inoculated with 0.01 ml. of a 48 hour culture of *reducing Achromobacter*; incubation was carried out at 30°C.

The cell suspension technique was similar to that previously employed except that 50 ml. flasks were used and anaerobic conditions were maintained by passing oxygen-free nitrogen through the solution. Four series were prepared, each containing lactic acid, trimethylamine oxide and bacterial suspension; to each of three series were added small amounts (final concentration 0.002 M.) of methylene blue, nitrate or fumaric acid; and the fourth kept for control.

The results are expressed graphically in figures 3 and 4. Figure 3 shows the influence of the acceptors on the reduction of the oxide during the growth of the organism. In the presence of methylene blue there was an inhibitive effect until the dye was partially reduced at (C); it is important, however, to observe that the methylene blue was not completely reduced until considerable oxide was

reduced at (D). The nitrate produced an inhibiting influence similar to the methylene blue. Spot test for nitrite showed that the nitrate was reduced immediately at (A); tests indicating no further reduction were obtained at (B). On the other hand, fumarate did not exhibit this inhibiting phenomenon, that is, the reduction of the oxide in the fumarate series and in the control series proceeded simultaneously.

The results plotted in figure 4, obtained by utilizing cell suspensions, confirm those of figure 3. Methylene blue completely inhibited the reduction of the oxide for the first 30 minutes of incubation; at this point (C), however, the initial reduction of the methylene blue occurred and the oxide was reduced; complete reduction of the dye was observed at (D). Nitrates were immediately reduced, starting at (A); no further reduction could be observed at (B). Fumarate, as was observed in the growth experiment, followed the control for the first two hours; at this point, the cells activated it to function catalytically. Quastel, Stephenson, and Whetham (1925) have reported fumarate acting not only as a hydrogen acceptor, but also as an oxygen acceptor becoming a carbon source. Thus, it is possible to explain the catalytic action of fumarate in the light of this consideration.

This inhibiting phenomenon was not a result of attenuated growth or activity of the cell. Growth was as prolific in the presence of methylene blue and nitrate as that observed in the control. It should be recalled that the reduction of the oxide occurred before the methylene blue was completely reduced; thus, as concluded by Yudkin (1935) and discussed by Watson (1939), such a phenomenon is not directly related to the potential of the environment but to cell surface activities. The evidence points to a poising influence on these cell activating mechanisms. As discussed by Cannan, Cohen, and Clark (1926) cells deprived of oxygen develop progressively a more negative potential. In this course they traverse in order the zones characteristic of reversible indicators; thus in one series of the present experiment methylene blue was present, causing the potentials at the cell surface to pass into equilibrium with the dye system. But as the dye was progressively reduced the potential developed at the cell surfaces—not necessarily in the environment as a whole—traversed to a lower potential, thereby reducing trimethylamine oxide.

Since nitrate produced an inhibiting effect similarly to methylene blue, it is apparent that irreversible systems are able to develop equilibrium potentials comparable to reversible systems. The fact that fumarate did not inhibit the reduction of the oxide throws further light on the phenomenon. It is shown in a subsequent experiment that fumarate can be activated as a hydrogen acceptor in a manner comparable to trimethylamine oxide in the anaerobic growth of *reducing Achromobacter*. These results indicate that the failure of fumarate to inhibit the reduction of the oxide as did the nitrate was not because of the inability of the organism to activate this acceptor. If the potentials set up at cell surfaces pass into equilibrium with potentials of irreversible systems of nitrate, fumarate and trimethylamine oxide in the sense of reversible systems as discussed by Cannan, Cohen, and Clark (1926) this phenomenon could be explained by the relative position of these compounds in the potential series. In other words, fumarate was not inhibitive because it is lower than trimethylamine oxide in the potential series,

while it is possible that nitrate is higher than the oxide in the series and was therefore an effective inhibitor. It must be understood, however, that while these results are in support of a chemical potential theory they are not conclusive. Meldrum (1934) pointed out that certain systems reduced methylene blue but not quinone, and suggested that it is not a case of chemical potential but rather

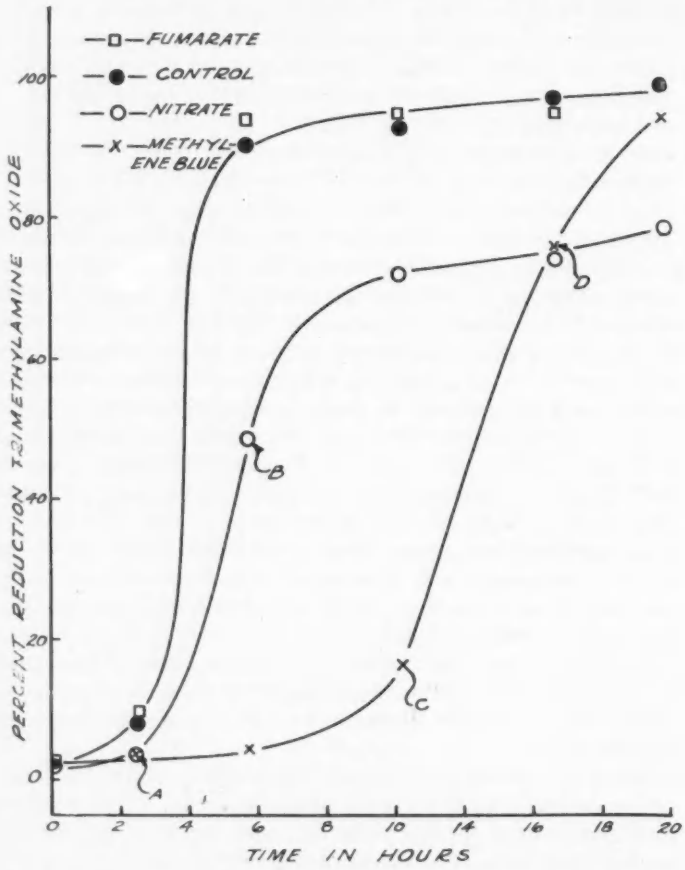

FIGURE 3. Influence of hydrogen acceptors on reduction of trimethylamine oxide during the growth of *reducing Achromobacter*.

of mechanism. A further study of this phase, including the determination of the apparent reduction potential of trimethylamine oxide, should throw some additional light on the problem.

To support the conclusions derived previously, it was necessary to determine the ability of fumarate to function as a hydrogen acceptor in the anaerobic growth of *reducing Achromobacter*.

The principle of the method was similar to that used in the previous paper, based on the ability of hydrogen acceptors to support growth anaerobically. The

same technique was employed adding fumaric acid in the same molecular concentration as trimethylamine oxide. The experiment was adequately controlled employing fumarate-free media. As a further control, trimethylamine oxide and oxygen were also employed as hydrogen acceptors. The counts were made from a uniform suspension of *reducing Achromobacter*.

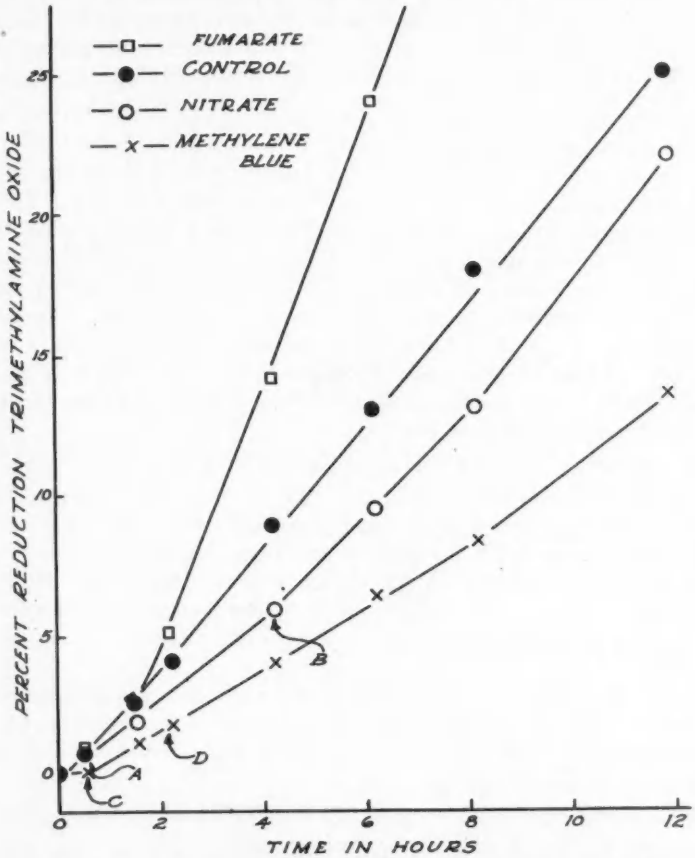

FIGURE 4. Influence of hydrogen acceptors on reduction of trimethylamine oxide in the presence of cell suspensions of *reducing Achromobacter*.

The results are recorded in table II. Since these counts check within the error of the method it is possible to conclude that fumarate functions as a hydrogen acceptor comparable to trimethylamine oxide and oxygen.

TABLE II. A comparison of fumarate, trimethylamine oxide, and oxygen as to their utilization as hydrogen acceptors in the growth of *reducing Achromobacter*.

Acceptor	Fumarate		Trimethylamine oxide		Oxygen	
Bacteria (no. per ml. $\times 10^9$).....	18.0	16.6	15.5	16.0	18.8	16.0
	16.5	14.5	15.8	14.0	15.8	16.5

QUANTITATIVE RELATIONSHIP

It has been suggested earlier in the paper that trimethylamine oxide in solution at the cell surface becomes activated in the sense of Quastel (1926); in other words, if there is a suitable hydrogen donator present such as lactic acid, it becomes activated by the dehydrogenases; the hydrogen from the donator is transferred to and accepted by the oxygen from the oxide. Thus the first step in the reaction is as follows:—

$$CH_3CHOHCOOH + (CH_3)_3NO \rightarrow CH_3COCOOH + (CH_3)_3N + H_2O.$$

If this reaction does take place, pyruvic acid should be present in the reaction solution. In several preliminary experiments, tests were carried out for pyruvic acid, employing the method of Jowitt and Quastel (1937) based on the formation of its 2-4 dinitrophenylhydrazone; a positive test was never obtained. The reason for these negative tests is obvious on examining figure 2; it will be observed that pyruvate was oxidized at a greater rate than lactate as measured by the reduction of trimethylamine oxide. As lactic acid was oxidized, therefore, the pyruvate formed was also oxidized at a rate comparable to its production. The difficulty in finding pyruvate as an end product in reaction solution has been a common observation among other workers (Anderson 1938). Preliminary tests on the relation of oxide to lactate revealed the production of carbon dioxide. From these findings the reaction was considered to be:

$$CH_3COCOOH + (CH_3)_3NO + H_2O \rightarrow CH_3COOH + (CH_3)_3N + CO_2 + H_2O.$$

In the sense of Wieland (Krebs 1937) it is essential for water to take part in this reaction; in other words, a hypothetical hydrate is formed. The oxygen from the trimethylamine oxide is not accepted by the ketonic group from the pyruvate, but it accepts the hydrogen from the hydrate to form water. The validity of the participation of water is substantiated when the oxidation of pyruvate takes place in the presence of fumarate which in turn accepts the hydrogen to form succinate (Krebs 1937).

Employing the methylene blue technique, it has been proved that *reducing Achromobacter* cannot utilize acetate as a hydrogen donator. Assuming, therefore, that carbon dioxide is not activated as hydrogen acceptor the equation may be written:

$$CH_3CHOHCOOH + 2(CH_3)_3NO + H_2O \rightarrow CH_3COOH + 2(CH_3)_3N + CO_2 + 2H_2O.$$

To prove the validity of the above equation quantitative determinations were made on the reactants and two end products after the completion of the reaction. It was necessary that the reaction be carried out in an oxygen-free environment; a reaction tube was designed, therefore, based on the inverted U-tube of Quastel and Whetham (1924). A sketch of the tube is shown in figure 5. The two chambers are connected so that after the replacement of the oxygen with nitrogen the contents of each chamber can be mixed. This apparatus has been employed for the methylene blue technique as described earlier.

It was necessary to control this experiment thoroughly, since it has been shown that there is a slight reduction of the oxide in the absence of the donator. Wilson (1938) attributed this phenomenon to a slight endogenous metabolism.

Three reaction tubes, therefore, were employed, one containing all the reactants, and the remaining two acting as controls. The first tube was prepared by adding a bacterial suspension of *reducing Achromobacter* in arm A (figure 5); in the other arm was placed a solution of trimethylamine oxide and a quantity of lactic acid in excess of that required to reduce the oxide completely. These solutions were buffered at a hydrogen-ion concentration of pH 6.9. The final concentration of bacteria was of the order of 4×10^9 cells per ml. The controls were prepared in the same manner; the first control contained lactic acid but no trimethylamine oxide; the second control contained trimethylamine oxide but no lactic acid. The chambers were evacuated and filled with oxygen-free nitrogen. After the oxygen had been completely removed the bacterial suspension in chamber A was placed in chamber B with the reactants. The reaction solutions were analyzed after 48 hours' incubation at 25°C.

Lactic acid was determined by the method of Friedemann and Graeser (1933). The Beatty and Gibbons (1937) modification of the Parnas-Mozolowski apparatus

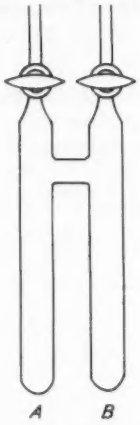

FIGURE 5. Sectional view of the reaction tube.

was employed for the trimethylamine determinations. Trimethylamine oxide was determined by Lintzel's (1934) method and carbon dioxide determinations were made in the Van Slyke volumetric apparatus. The average deviation between duplicate determinations has been recorded; it will be observed that these measurements are within 3 per cent.

The results of the analyses are recorded in table III. Since the trimethylamine oxide was completely reduced, the value 124 mg. per cent was placed in the completed equation and the theoretical values for the remaining reactant and end products calculated.

In the following, the theoretical values are compared with the values found by analysis:

	Lactic acid	Carbon dioxide	Trimethylamine
Theory	74.5 mg. %	36.4 mg. %	98.0 mg. %
Found	78.0 mg. %	34.2 mg. %	94.0 mg. %
(per cent of theory,	104.5	94.0	96.0)

These results confirm the theoretical equation within the limits of the methods. Since the equation held, the following general equation will express the reduction of trimethylamine oxide by *reducing Achromobacter* in the presence of an oxidizable substrate:—

$$AH_2+(CH_3)_3NO\rightarrow A+(CH_3)_3N+H_2O.$$

AH_2 is the oxidizable subtrate or hydrogen donator, oxidized in the presence of trimethylamine oxide to form the oxidized substrate A plus trimethylamine and water.

TABLE III. Reactants and end-products in the oxidation-reduction reaction between lactic acid and trimethylamine oxide in the presence of *reducing Achromobacter*.

$(CH_3)_3N=O$ (mg. % in lactic acid free control)	$(CH_3)_3N=O$ (mg. % due to lactic acid)	$(CH_3)_3N=O$ (mg. % reduction due to lactic acid)
124.0±2.0	−2.5±2.5	124.0
Lactic acid (mg. % in oxide free control)	Lactic acid (mg. % in presence of oxide)	Lactic acid (mg. % reduction due to oxide)
348.5±2.5	270.5±3.5	78.0
CO_2 (mg. % in presence of lactic acid +oxide)	CO_2 (mg. % in oxide free control)	CO_2 (mg. % due to presence of oxide)
36.6±0.0	2.4±0.4	34.2
$(CH_3)_3N$ (mg. % in presence of lactic acid +oxide)	$(CH_3)_3N$ (mg. % in lactic acid free control)	$(CH_3)_3N$ (mg. % due to presence of lactic acid)
107.4±1.3	13.4±0.1	94.0

It should be recalled that of the five reactants and end products comprising the derived equation only four were determined experimentally; the fifth, acetic acid, has only mathematical confirmation. Because of the small quantities of the solutions available for analysis accurate determinations for acetic acid were not achieved.

GENERAL DISCUSSION

A comparative study of the dehydrogenase activity between *reducing* and *non-reducing Achromobacter* employing methylene blue and trimethylamine oxide as hydrogen acceptors has shown a further relationship between their respiratory

mechanisms. It is apparent, as formerly suggested, that the difference between the above groups is one of degree rather than kind; in other words, *non-reducing Achromobacter* have lost their ability to activate trimethylamine oxide as a hydrogen acceptor, probably as a result of the stimulus of the environment in the sense of Knight (1936).

The trimethylamine oxide *sparing effect* as observed by Watson (1939) on aerating fish muscle press juice has been demonstrated by the addition of small concentrations of hydrogen acceptors such as methylene blue, nitrate and fumarate; the latter failed to inhibit the reduction of the oxide. Since fumarate, however, was readily utilized in the anaerobic growth of *reducing Achromobacter*, there was evidence of a preferential activation of hydrogen acceptors, either as a function of their place in the potential series or as a function of mechanism. If the influence of these hydrogen acceptors is a function of their apparent reduction potential it is evident that trimethylamine oxide is slightly higher in the series than fumarate and lower than nitrate. The observation of Watson (1939) points to this latter relationship in that certain *Flavobacter* reduced nitrates readily but failed to reduce trimethylamine oxide. It has been recorded by Zobell (1932) that *Thiobacillus denitrificans* loses much of its reducing ability when cultivated under aerobic conditions; this phenomenon is apparently related to the oxide *sparing effect* and the adaptation hypothesis as presented in the previous paper.

The quantitative study of the coupled oxidation-reduction reaction involving trimethylamine oxide as hydrogen acceptor and lactic acid as the donator gave strong evidence for the following general equation:—

$$AH_2+(CH_3)_3NO\rightarrow A+(CH_3)_3N+H_2O.$$

where AH_2 is the oxidizable substrate or hydrogen donator and A the oxidized substrate. Variations in dehydrogenase activity between the strain of *reducing Achromobacter* employed in the present experiment and other strains may produce discrepancies in the reaction between lactic acid and trimethylamine oxide as recorded in this paper. Regardless of possible differences in dehydrogenations, however, it is obvious that the principle of the reduction of trimethylamine oxide will hold according to the general equation.

Since Sharp (1934, 1935) has shown an accumulation of 0.40 to 0.45 per cent lactic acid in fish muscle and Beatty and Collins (unpub.) have shown lactic acid to disappear concomitantly with the production of trimethylamine in cod muscle press juice, the significance of the results as recorded in this paper becomes obvious. We have seen that two molecules of trimethylamine oxide were reduced for each molecule of lactic acid oxidized. Thus, considering the quantity of carbohydrates and their derivatives present in fish muscle it is evident that the bacteria present can reduce the total concentration of trimethylamine oxide employing only these substances as hydrogen donators. It is possible to infer, therefore, that trimethylamine produced in cod muscle press juice is a result of energy yielding reactions between trimethylamine oxide and appropriate hydrogen donators, especially carbohydrates and their derivatives, during the anaerobic respiration of *reducing Achromobacter*. These conclusions confirm the conception of fish spoilage in the sense of Beatty and Collins (unpub.).

SUMMARY

Trimethylamine oxide is reduced as a linear function of time by cell suspensions of *reducing Achromobacter* in the presence of a number of hydrogen donators, namely, glucose, glycogen, lactate, and pyruvate.

Suspensions of *non-reducing Achromobacter* are unable to activate trimethylamine oxide as hydrogen acceptor in the presence of the above donators.

Reducing and *non-reducing Achromobacter* show the same dehydrogenases as tested by the methylene blue technique in the presence of a number of different hydrogen donators. A further relationship between the groups is discussed.

The addition of small concentrations of hydrogen acceptors such as methylene blue and nitrate inhibits the reduction of trimethylamine oxide. Since fumarate is not inhibitive, but, like trimethylamine oxide, supports the anaerobic growth of *reducing Achromobacter*, there is evidence of a preferential activation of hydrogen acceptors.

A coupled oxidation-reduction reaction between lactic acid and trimethylamine oxide is tested quantitatively; a general equation involving the reduction of trimethylamine oxide in the presence of a hydrogen donator and *reducing Achromobacter* is derived from the results.

The results as applied to a conception of fish spoilage are discussed.

ACKNOWLEDGEMENT

As in the foregoing paper the author wishes to express his thanks to Dr. D. B. Finn, Dr. S. A. Beatty and Dr. E. Hess for their many helpful suggestions and constructive criticism. The excellent technical assistance and interest given by Mr. William Leahy was greatly appreciated.

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The Mysidacea of Eastern Canadian Waters

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ABSTRACT

Records of occurrence of nine different species out of thirteen known in the region, including *Boreomysis tridens*, not known previously.

I am indebted to Professor A. G. Huntsman for the opportunity of examining small collections of Mysidacea made in Canadian waters during various Canadian fisheries expeditions. In the following pages records of Mysidacea are included from the "*Acadia*", "*Princess*" and "*33*" expeditions of 1915, the Cheticamp and Miramichi expeditions in the "*Prince*" during 1917, and the Belle Isle Strait expedition in the "*Arleux*" in 1923. In addition to this material there are specimens from the vicinity of the Atlantic Biological Station, St. Andrews, N.B., and Mrs. A. B. Needler has been good enough to send me specimens collected in the neighbourhood of the Prince Edward Island Biological Station of the Canadian Fisheries Research Board. These various collections include in all nine species out of the thirteen now known from Canada. None of the species is new to science but one of them, *Boreomysis tridens* G. O. Sars, has not been recorded previously from Canadian waters. All species are included with a complete list of references to all literature dealing with this area. This note, therefore, summarises all that is known of the Mysidacea of Eastern Canadian waters.

It will be observed that the list of species is somewhat meagre. Particularly may this be said of shore and shallow water forms. Only two littoral species are included in the material, *Mysis stenolepis* S. I. Smith and *Neomysis americana* (S. I. Smith), though to judge from the collections sent from the Biological Station, these species are abundant in shallow water. The deeper waters will probably yield a richer harvest of both bottom living and pelagic species when more thoroughly and systematically explored. In the recent paper by Stephensen (1933) will be found a full list of the species of West Greenland, and any or all of those not yet found may be expected to occur in the deeper waters of Canada.

The Mysidacean fauna of eastern Canada as a whole includes, first of all, one freshwater species, *Mysis relicta*, Lovén, which is circumpolar in distribution in the fresh waters of Canada and Northern Europe. It is a relict of Arctic origin. Two further species, *Mysis stenolepis* Smith, and *Neomysis americana* (Smith) are littoral species not known from localities further north than the gulf of St. Law-

rence, but extending southwards into the shallow waters of the United States. They belong to northern genera but the species are confined to this small area of the coastline. The remaining forms are deeper water species of Arctic origin, more or less circumpolar in distribution, which extend down into the boreal part of the west Atlantic at least as far as the coasts of the United States. The warm water of the Atlantic ocean, which, under certain conditions, penetrates into the gulf of St. Lawrence, is known to bring with it certain warmer water species of Euphausians. No temperate or warmer water species of Mysidacea have as yet been detected as a result of this water movement, but the records of *Boreomysis microps* G. O. Sars, *Longithorax fuscus* Hansen, and species of *Gnathophausia* and *Eucopia* from West Greenland by Stephensen (1933) lead one to anticipate that such species may eventually be found in Canadian waters.

Under each species I have given references to Canadian literature only, and the distribution refers only to the waters of the eastern coasts of America.

Boreomysis arctica (Kröyer). (Stephensen 1933).

Distribution. West Greenland; entrance to Hudson strait; off the eastern coast of New England.

Boreomysis nobilis G. O. Sars. (Stephensen 1933).

Material examined. C.G.S. "*33*": Sta. 57, off the bay of Islands, Nfld., 9.8.1915, young fish trawl, 210 m., sixteen specimens up to 45 mm. C.G.S. "*Arleux*": Sta. 89, bay of Exploits, Nfld., 12.9.1923, vertical tow net, 300-0 m., six females, the two largest of which measure 35 mm. and are carrying eggs in the brood pouch.

Distribution. West Greenland; Jones sound; Lancaster sound.

Boreomysis tridens G. O. Sars.

Material examined. C.G.S. "*33*": Sta. 23, 49°03′N., 63°58′W., 25.6.1915, trawl, 355 m., one.

Distribution. West Greenland; east coast of New England.

Erythrops erythrophthalma (Goës) [Calman 1901 (*E. microphthalma*) (?); Gardiner 1934].

Material examined. C.G.S. "*Princess*": Sta. 50, 46°18′N., 61°59′W., 12.8.1915, tow net, 40-0 m., three females and two males. C.G.S. "*33*": Sta. 55, off the bay of Islands, Nfld., 7.8.1915, tow net, 110-0 m., twenty-seven; Sta. 57, off the bay of Islands, 9.8.1915, young fish trawl, 210 m., twenty-two; Sta. 59, inside South head, bay of Islands, 10.8.1915, tow net, 270-0 m., thirteen. C.G.S. "*Arleux*": Sta. 14, Mingan channel, gulf of St. Lawrence, 11.8.1923, vertical tow net, 150-0 m., one adult male, 14 mm.; Sta. 23, off Flat island, Que., 15.8.1923, vertical tow net, 230-0 m., one male; Sta. 24, off St. Paul bay, Nfld., 16.8.1923, vertical tow net, 90-0 m., one immature; Sta. 51, N.E. of Belle isle, 19.8.1923, vertical tow net, 230-0 m., two males and twenty-two immature; Sta. 59, bay of Islands, Nfld., 30.8.1923, vertical tow net, 275-0 m., four immature females,

11 mm.; Sta. 83, off Fichot islands, Nfld., 11.9.1923, vertical tow net, 190-0 m., one immature female, 12 mm.; Sta. 101, off St. Pierre, 20.9.1923, vertical tow net, 150-0 m., one female, 14 mm.

Remarks. The eyes of these specimens are perhaps a little smaller than shown in Sars' figures, that is to say, the pigmented area in dorsal view is not quite as extensive as indicated by Sars, but the telson is certainly that of *E. erythrophthalma*, as distinct from *E. microphthalma*, in having the outer pair of spines on the apex not much more than half as long as the inner pair. Calman (1901) records *E. microphthalma* from 100 fathoms (183 m.) off Labrador. The specimen is no longer in existence, and I suggest, provisionally, that it belonged to the same species as those which I here refer to *E. erythrophthalma*.

Distribution. West Greenland; Labrador; Nova Scotia; east coast of United States.

Meterythrops robusta Smith. (Whiteaves 1874; Smith 1879; Whiteaves 1901; Kindle and Whittaker 1918; Stephensen 1933).

Material examined. C.G.S. "*33*": Sta. 59, inside South head, bay of Islands, Nfld., 10.8.1915, tow net, 270-0 m., one male, one female and one immature. C.G.S. "*Arleux*": Sta. 59, bay of Islands, 30.8.1923, vertical tow net, 275-0 m., one male, 22 mm., one female, 15 mm., and two immature specimens, 6 mm.

Distribution. West Greenland; Smith and Jones sounds; Chaleur bay; off the coast of New England.

Pseudomma truncatum Smith. (Whiteaves 1874; Smith 1879; Whiteaves 1901; Kindle and Whittaker 1918; Stephensen 1933).

Material examined. C.G.S. "*33*": Sta. 55, off the bay of Islands, Nfld., 7.8.1915, tow net, 110-0 m., thirty; Sta. 57, off the bay of Islands, 9.8.1915, young fish trawl, 210 m., fifty-three; Sta. 59, inside South head, bay of Islands, 10.8.1915, tow net, 270-0 m., five. C.G.S. "*Arleux*": Sta. 23, off Flat island, Que., 15.8.1923, vertical tow net, 230-0 m., one female, 12 mm.; Sta. 59, bay of Islands, Nfld., 30.8.1923, vertical tow net, 275-0 m., one adult male, 15 mm., one immature female, 10 mm.; Sta. 94, off cape Bonavista, Nfld., 15.9.1923, vertical tow net, 350-0 m., one adult male and one adult female, 14 mm.

Distribution. West Greenland; Lancaster sound; off Chaleur bay.

Pseudomma roseum G. O. Sars. (Whiteaves 1874; Smith 1879; Whiteaves 1901; Kindle and Whittaker 1918).

Distribution. West Greenland; off Gaspé; off the coast of New England.

Stilomysis grandis (Goës). [Rodger 1895 (*Mysideis grandis*); Calman 1901].

Distribution. West Greenland; Labrador; strait of Belle Isle.

Remarks. I have examined the specimen recorded in Calman (1901). It is now much damaged, but, as far as can be seen under the circumstances, I can confirm the identification. It seems, moreover, more than likely that it is one of the specimens which Rodger himself recorded from the strait of Belle Isle in 1895.

Mysis mixta Lilljeborg. (Smith 1879; Kindle and Whittaker 1918; Stephensen 1933).

Material examined. C.G.S. "*Acadia*": Sta. 5, 43°56′N., 61°32′W., 30.5.1915, tow net, 60-0 m., one female; Sta. 59, 43°48′30″N., 60°50′W., 25.7.1915, tow net, 25-0 m., two. C.G.S. "*Princess*": Sta. 33, 48°17′N., 62°54′W., 4.8.1915, tow net, 30-0 m., three, and tow net 80-0 m., three; Sta. 40, 50°05′N., 61°16′W., 5.8.1915, tow net, oblique haul, two, tow net, 30-0 m., one, and tow net, 70-0 m., three; Sta. 50, 46°18′N., 61°59′W., 12.8.1915, tow net at surface, seventeen, mostly juvenile, and tow net, 40-0 m., one immature. C.G.S. "*33*": Sta. 55, off bay of Islands, Nfld., 7.8.1915, tow net, 110-0 m., four. C.G.B. "*Prince*": Sta. 31, 46°54′36″N., 61°15′30″W., 13.9.1917, tow net, 58-0 m., one; Sta. 77, 47°10′38″N., 64°57′05″W., 7.8.1918, tow net, 8-0 m., two. C.G.S. "*Arleux*": Sta. 75, south of Belle Isle, at entrance to strait, 7.9.1923, tow net, 20-30 m., one male, 27 mm.; Sta. 76, south of Belle Isle, at entrance to strait, 7.9.1923, tow net at surface, one male, immature, 18 mm.; Sta. 77, south of Belle Isle, at entrance to strait, 7.9.1923, tow net, 20-30 m., one male and one female 25 mm.; Sta. 89, bay of Exploits, Nfld., 12.9.1923, vertical tow net, 300-0 m., one female, 25 mm.

Distribution. West Greenland; Davis strait; Baffin bay; Exeter sound (Baffin Land); bay of Fundy; coasts of New England.

Mysis stenolepis Smith. (Smith 1879; Whiteaves 1901; Schmitt 1904; Kindle and Whittaker 1918).

Material examined. Atlantic Biological Station, St. Andrews, N.B.: Upper Birch cove ponds, 28.8.1933, six; Tidal cove, outer pond, 28.8.1933, dip net, two hundred; around rocks at low tide, 31.8.1933, fifty-five; from ledges near the station, 8.10.1933, fifty. *Prince Edward Island Biological Station*: Hog island reef, Malpeque bay, 8.8.1935, two; Malpeque bay, 14.9.1935, bottom tow net, 10 ft. (3 m.), two. I have also seen one female specimen caught at Trois Pistoles, Que., in pools among *Zostera* in July, 1929, by Professor Georges Préfontaine, and submitted to me by the United States National Museum.

Distribution. Anticosti island; Halifax; eastern coast of the United States.

Mysis oculata (Fabricius). [Adams 1852 (*M. flexuosa*); Walker 1862 (*M. flexuosa*); Bell 1855 (*M. fabricii*); Packard 1863 (*M. spinulosa*); Dawson 1886 (*M. spinulosa*); Stimpson 1863; Packard 1867; Miers 1877 and 1878; Smith 1879; Smith 1884 and 1885; Dawson 1886; Whiteaves 1901; Stafford 1912; Kindle and Whittaker 1918; Schmitt 1919; Hjort and Ruud 1929; Stephensen 1933; Rathbun 1909 (*Mysis* sp.); Fewkes 1888 (*M. rayii*)].

Material examined. C.G.S. "*33*": Sta. 55, off the bay of Islands, Nfld., 7.8.1915, tow net, 110-0 m., five; Sta. 57, off the bay of Islands, Nfld., 9.8.1915, young fish trawl, 210-0 m., eight females. C.G.S. "*Princess*": bay of Islands, Nfld., 11.8.1915, in practically fresh water at the mouth of the river Humber, eighty-six young specimens.

Distribution. West Greenland; Davis strait; northern and arctic Canada; Labrador; Gaspé.

Mysis relicta Lovén. [Smith 1871; Smith and Verrill 1871; Stimpson 1871; Smith 1874; Kellicott 1878; Underwood 1886; Ward 1896; Rathbun 1909; Pearse 1910; Huntsman 1913 and 1915; Ortmann 1918; Schmitt 1919; Clemens and Bigelow 1922; Johansen 1922; Clemens and others 1923; Bajkov 1930; Hoy 1872 (*M. diluvianus*)].

Distribution. Bernard harbour (arctic Canada); lakes from lake Winnipeg to lake Ontario.

Neomysis americana (Smith). (Fish and Johnson 1937).

Material examined. Atlantic Biological Station, St. Andrews, N.B.: near the station at low tide, 31.8.1933, several hundreds. *Prince Edward Island Biological Station*: Hog island reef, Malpeque bay, 28.6.1933, bottom tow net, large numbers; 27.7.1933, surface, several; 8.8.1935, tow net at 25 ft. (7.6 m.), about two hundred; Curtain island reef, Malpeque bay, 4.8.1933, tow net at night, several; middle of inlet near station, 14.9.1935, bottom tow net at 10 ft. (3 m.), fifty; Claude Williams creek, Bideford river, 14.9.1935, bottom tow net at 8 ft. (2.4 m.), ten.

I have also seen a male and female specimens caught at Trois Pistoles, in pools among *Zostera*, in July, 1929, by Professor Georges Préfontaine, and submitted to me by the United States National Museum.

Distribution. Eastern coast of the United States, in shallow water.

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Larval Life of the Oyster (*Ostrea virginica*) in Bideford River

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ABSTRACT

Oysters spawn when ripe, with rising temperatures that may or may not reach 20°C. and at times not determined by lunar cycles. The growth of the larva to the ultimate size, height 365μ, at temperatures of 19, 20 and 21°C., requires 30, 26 and 24 days respectively. The growth curves developed, not sigmoidal in shape, have been used to predict spatfall maxima.

INTRODUCTION

It has long been known that in many districts maxima may be observed in the rate of settlement of oyster larvae. Various authors (see Prytherch 1928) have shown that this is due to the presence of larval age groups established by distinct spawning bursts and to the maturation of each group to the settling stage practically as a unit. Other than Sherwood's 1930 study (unpublished) a close examination of these phenomena in Canadian waters had never been made previous to 1936, when this two-years' investigation was undertaken on the suggestion of Dr. A. W. H. Needler at the Prince Edward Island Biological Station.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. H. J. Van Cleave, Professor of Zoology of the University of Illinois, and Dr. A. W. H. Needler, in charge of the Prince Edward Island Biological Station, for their kindly direction of this study during the two winters and summers respectively. The assistance of Dr. A. B. Needler, who made the results of her studies of oyster gonads available, is likewise gratefully acknowledged. The author is indebted to the Fisheries Research Board of Canada and the Zoological Laboratory of the University of Illinois for facilities provided during the investigation. The present paper constitutes Contribution No. 527 of the Zoological Laboratory of the University of Illinois.

METHODS

Working out the larval history required that the spawning dates be detected, the progress of larval age groups followed and the length of the larval periods determined.

DETERMINATION OF SPAWNING DATES

By examination of oyster gonads at regular intervals, noting changes in their size and general appearance, spawning dates were approximately determined. When it was certain that spawning had taken place in the interval between two

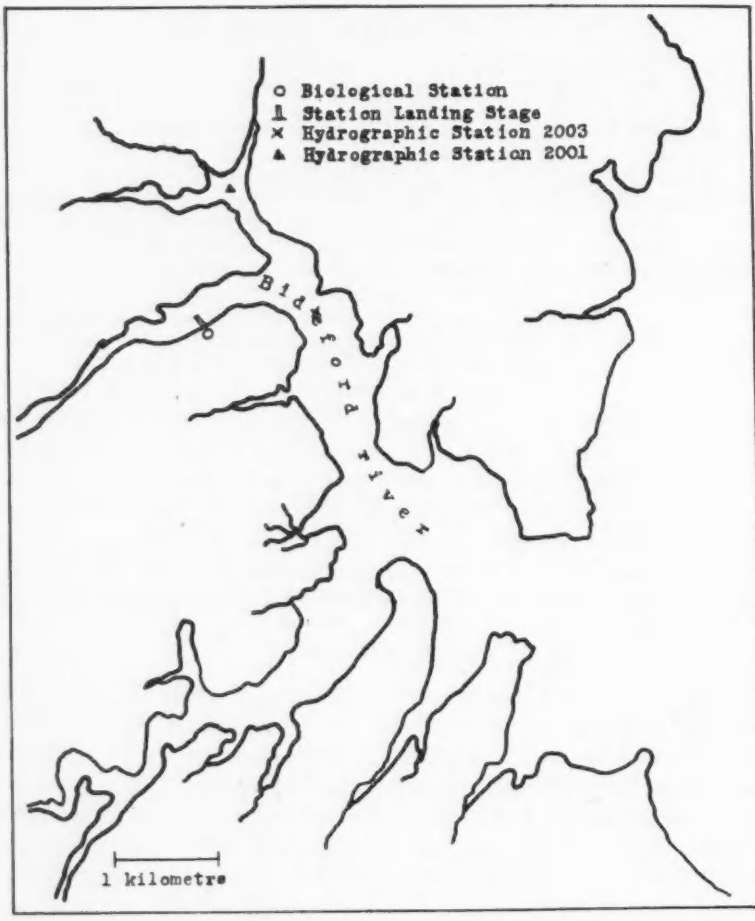


FIGURE 1. Bideford river district.

examinations, it was usually possible to determine the exact date by reference to temperature records. This is because spawning ordinarily requires a rising temperature in the neighbourhod of 20°C. for its initiation (Galtsoff 1938). In the present calculations spawning is assumed to have taken place on the first day within the interval, on which there was such a rise, and that day is taken as the first of the brood's free-swimming period.

The temperature records of the Prince Edward Island Biological Station have been available. Readings are made twice daily (except on Sundays) at the end of the landing stage at the edge of the channel of Bideford river where the water is two to two and a half metres deep at half tide. Readings were made in 1936 at the surface only, but in 1937 at both surface and bottom.

TABLE I. Spawning of oysters in 1936.

Date	Effective spawning temperature (°C.)	Condition of gonads	Probable spawning dates*	Brood number
		June 8 Oysters immature		
June 10	22.3	June 10 No apparent spawning but some oysters mature		
June 14	20.0		June 14	I
		June 17 Some oysters may have spawned a little		
		June 22 Oysters all filled. Some may have spawned lightly		
June 24	18.7		June 24	II
		June 25 Shallow water oysters have spawned		
		June 29 Oysters either spawning or ready to spawn		
July 3	19.9		July 3	III
		July 6 Several oysters seem to have spawned		
July 8	20.9	July 8 Oysters have spawned heavily. Most are ¼ spent	July 8	IV
		July 13 Oysters about ½ spent		
		July 17 Oysters about ½ spent		
July 20	20.6		July 20	V
		July 27 Oysters about ¾ spent		
July 28	21.0		July 28	VI
Aug. 2	21.0		Aug. 2	VII

*These dates are determined partly from data in other columns and partly from the results of studies reported below. Each of these dates is indicated in figures 2 and 3 by an "S".

FOLLOWING THE BROODS

For this purpose samples of larvae were obtained in plankton tows with a net of number 18 bolting cloth. In 1936 they were taken on every second day at station 2001 (figure 1) where most of the commercial collectors were located, and in 1937 on every third day, opposite the landing stage.

Petersen's method of recognizing age groups by the frequencies of various sizes was used. The dimensions of camera lucida images of the larvae magnified 315 diameters were marked off along the lines of a ruled page and later converted into the equivalents for the larvae in micra.

Larval height, the distance from the tip of the umbone of the shell to the middle of its free margin, was used as the best index of the size, since in repeated measurements of the same individual it showed less variation than did other dimensions. A single height measurement usually fell within 10μ of the mode of a series of such values for the same larva.

TABLE II. Spawning of oysters in 1937

Date	Effective spawning temperature (°C.)	Condition of gonads	Probable* spawning dates	Brood number
June 8	21.3			
		June 9 Oysters filled with ripening spawn		
		June 14 Spawn in shallow-water oysters ripe		
June 15	20.5	June 15 Some spawning has taken place	June 15	I
		June 18 Oysters about $\frac{1}{4}$ spent		
June 20	19.1		June 20	II
		June 22 Oysters $\frac{1}{4}$ to $\frac{1}{2}$ spent		
June 26	20.3		June 26	III
June 29	20.1		June 29	IV
July 4	20.0		July 4	V
July 5	20.8	July 5 Oysters $\frac{3}{4}$ spent	July 5	V

*These dates are determined partly from data in other columns and partly from the results of studies reported below. Each of these dates is indicated in figures 2 and 4 by an "S".

DETERMINATION OF SETTLEMENT DATES OF LARVAE

To determine the end of the larval period for a brood the date of the settlement maximum was found by following the rate of spatfall. In 1936, glass slides measuring 5.1 by 7.6 centimetres were suspended as experimental collectors at different depths in chains from the station landing stage and from a float at station 2001. In 1937, oyster shells were substituted for the glass, exact counts were made with a binocular microscope, and, to give comparable results, the very same collectors were used over and over again, being cleaned each time.

RESULTS

The temperatures are plotted in figure 2, using the higher of the two daily readings. These data and Dr. A. B. Needler's records are used in tables I and II to determine the spawning dates.

TABLE III. Larval settlement in 1936 and 1937 on experimental collectors.

	1936 Observations	1937 Average catch per shell from counts on 10 shells
July 14	No spat	0
15	" "	3.4
16	" "	16.4
17	" "	4.9
18	" "	3.4
19	" "	4.9
20	" "	3.5
21	" "	5.9
22	" "	4.6
23	" "	5.0
24	" "	13.6
25	" "	14.1
26	" "	8.2
27	One spat 1.8×1.8 mm.* on a commercial collector	18.7
28	No spat	27.0
29	" "	13.4
30	" "	5.7
31	" "	1.1
Aug. 1	11 spat on 13 slides	0.9
2	No observation	1.5
3	" "	0.2
4	Increase of 2-3 per slide since August 1	0.3
5	No observation	0.2
6	No increase	1.1
7	No spat	1.5
8	" "	0.2
9	" "	0.2
10	" "	0.2
11	2 spat on 11 slides	0.2
12	A few more	0.1
13	More	0
14	A considerable set	0
15	Very heavy set	0.1
16	Very heavy set	0.2
17	A few more	0.4
18	5 spat on 11 slides	0.4
19	No observation	0.2
20	" "	0.3
21	3 spat on 7 slides	0.4
22	7 spat on 7 slides	0.5
23	No observation	0.6
24	41 spat on 9 slides	0.1
25	No observation	0.1
26	39 spat on 9 slides	0.3
27	Out of water, 26th to 27th (late)	0.2
28	No observation	0.1
29	12 spat on 7 slides	0.1
30	No spat	0

*According to previous studies on growth rate (Medcof unpub.) this spat must have settled about July 14 or 15.

Size-frequency studies were made on samples of larvae taken in 1936 on June 27, July 15, 20, 24, 29, August 4, 7, 12 and 20, and in 1937 on July 5, 8, 13, 16, 20 and 23. The results of the measurements, plotted for groups with a 10μ range, are given in the frequency polygons of figures 3 and 4.

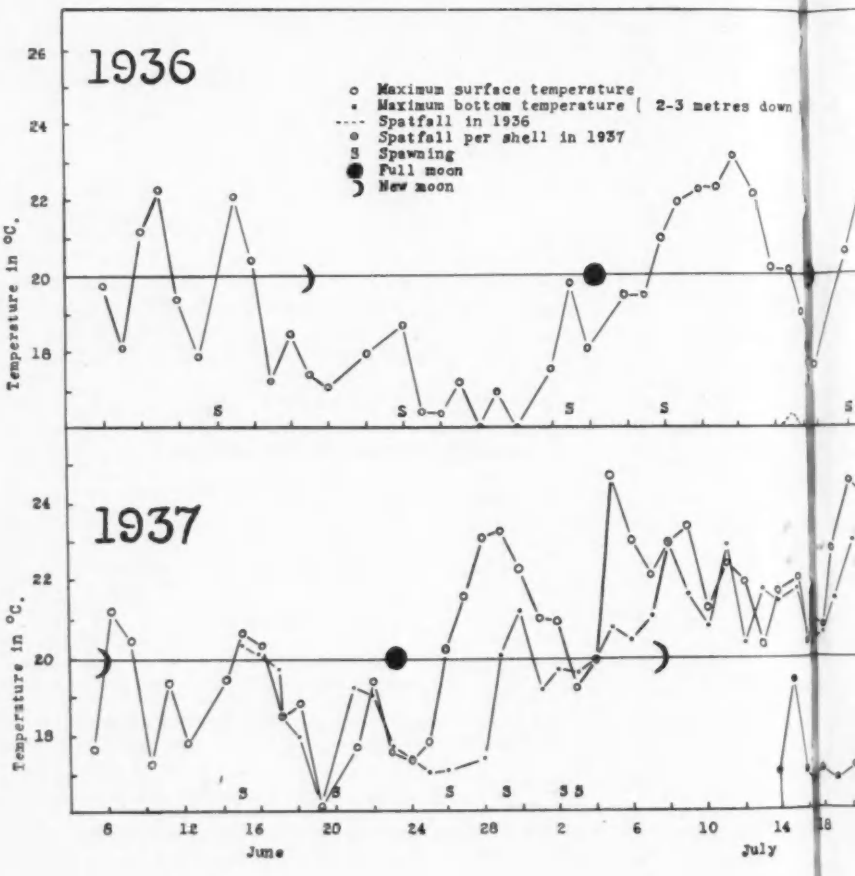
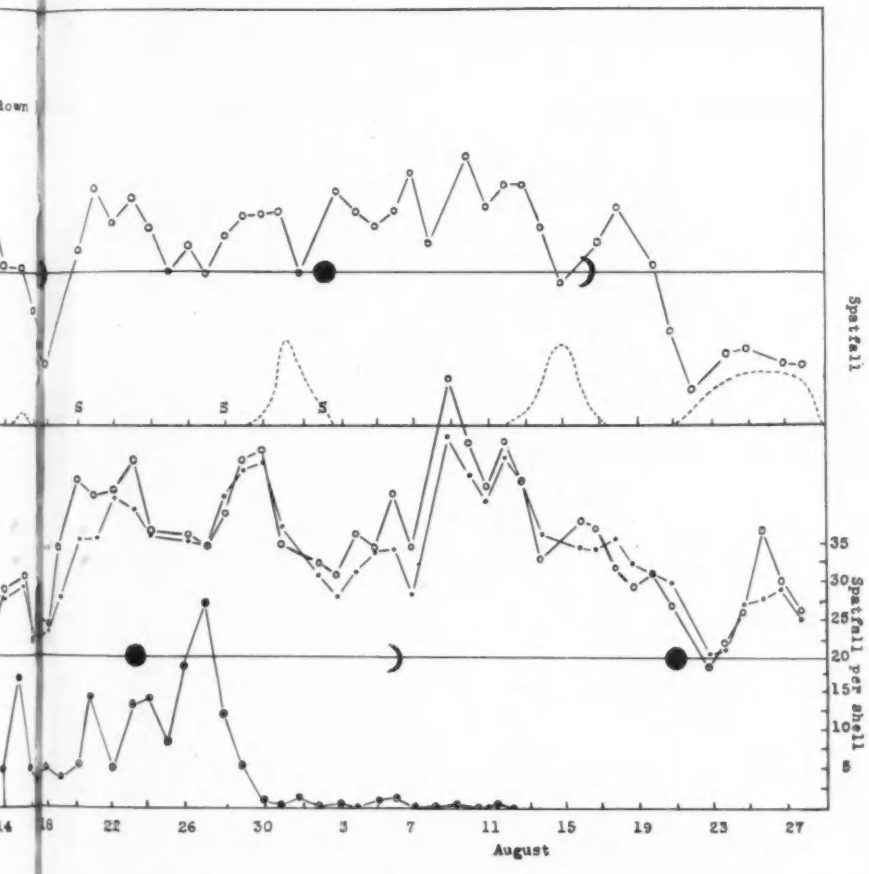


FIGURE 2. Moon phase, water temperature and spatfa

The data of spatfall have been summarized in table III and graphically represented in figure 2. The results of 1937 permit quantitative plotting, but the 1936 settlement maxima are indicated by "humps" whose heights are only roughly proportional to the extent of settlement that took place on the various days. With few exceptions these examinations were made at 9.00 a.m. so that the spat observed on any particular morning settled during the previous twenty-

four hours and rightly belong to the previous day. This correction was made in plotting the settlement data in figure 2.

Reliable mean values for the mature size of the Canadian larva have never been reported although various statements have been published from time to



ure 2 Spatfall at the landing stage of the biological station.

time (Stafford 1905, 1909, 1910, 1912; Nelson 1917). It is justifiable to take the dimensions of the prodissoconch of freshly-settled spat as a true measurement of this size since at settlement the prodissoconch growth ceases (Prytherch 1934).

For 32 spat freshly-settled on commercial collectors in 1936, the average diameters ranged from 280 to 370μ, but the measurements clustered closely about a modal value of 340μ. In 1937 more careful measurements of 194 fresh spat

showed much the same distribution with the mode for the height 365μ and for the length 335μ.

DISCUSSION AND RELATION OF RESULTS

Construction of Growth Curves

The positions of the modes of the size-frequency curves of figures 3 and 4

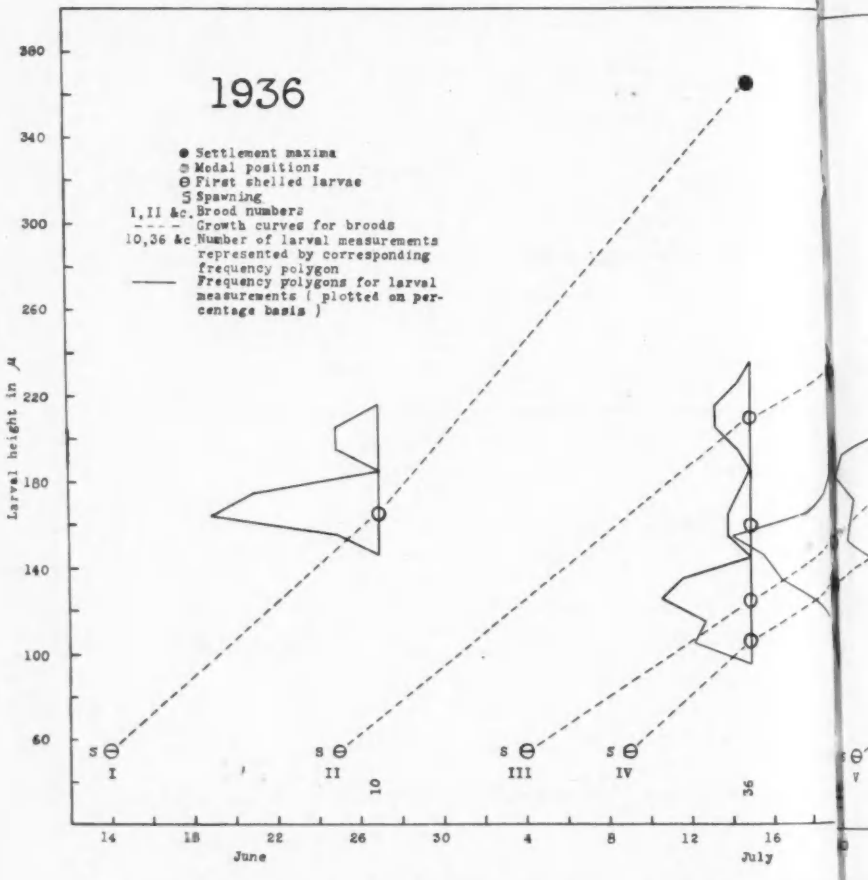
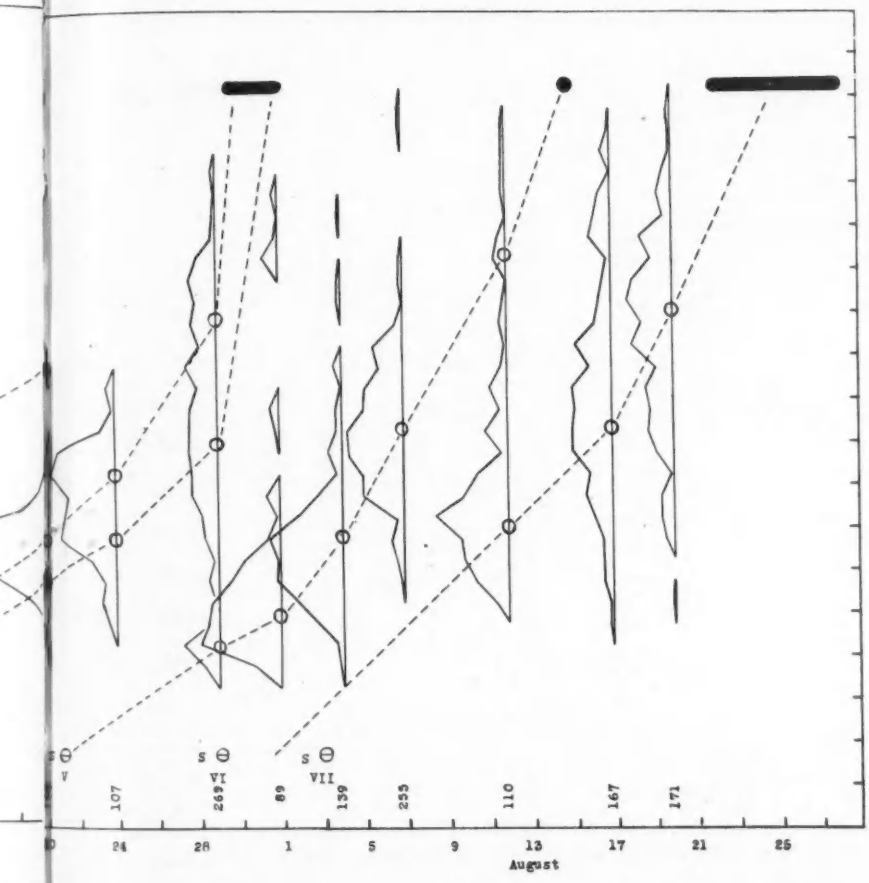


FIGURE 3. Size-frequency distribution of oyster larvae at different times in 1936, plott

were marked on the corresponding abscissae and these points joined to give partial growth curves for the various broods. These same curves were connected to points corresponding to size 55μ and times 1 day after each of the respective spawning dates. This starting point for the larvae is based upon Stafford's measurements (1912) of the earliest shelled larvae of the Canadian oyster, and Nelson's statement (1921) that the complete bivalve shell is formed within 24 to

36 hours after fertilization, if water temperatures remain about the level of the spawning threshold.

In order to relate settlement observations to larval growth, it is necessary to assume that once larvae reach their mature size, they settle without any considerable delay. This seems justified because no accumulations of large-sized



1936 plotted on a percentage basis and combined with spawning and settlement observations.

larvae previous to spatfall maxima were observed during plankton studies. This is in contrast to Schaefer's (1938) findings for *O. gigas*.

Inasmuch as the ultimate size was found to be the same in spite of considerable differences in the temperature conditions during the two years, it is reasonable to regard this maturation size as standard for all larvae. The growth curves may therefore be completed by extending them to another set of points corresponding

to height 365μ and times corresponding to the dates of the respective settlement maxima.

The growth curves for 1936 are on the whole well-defined because the spawnings occurred at widely spaced intervals. The complicated overlapping of the 1937 age groups obscures their boundaries and makes the study difficult. The marking of modal positions, as a result, is sometimes very arbitrary. The con-

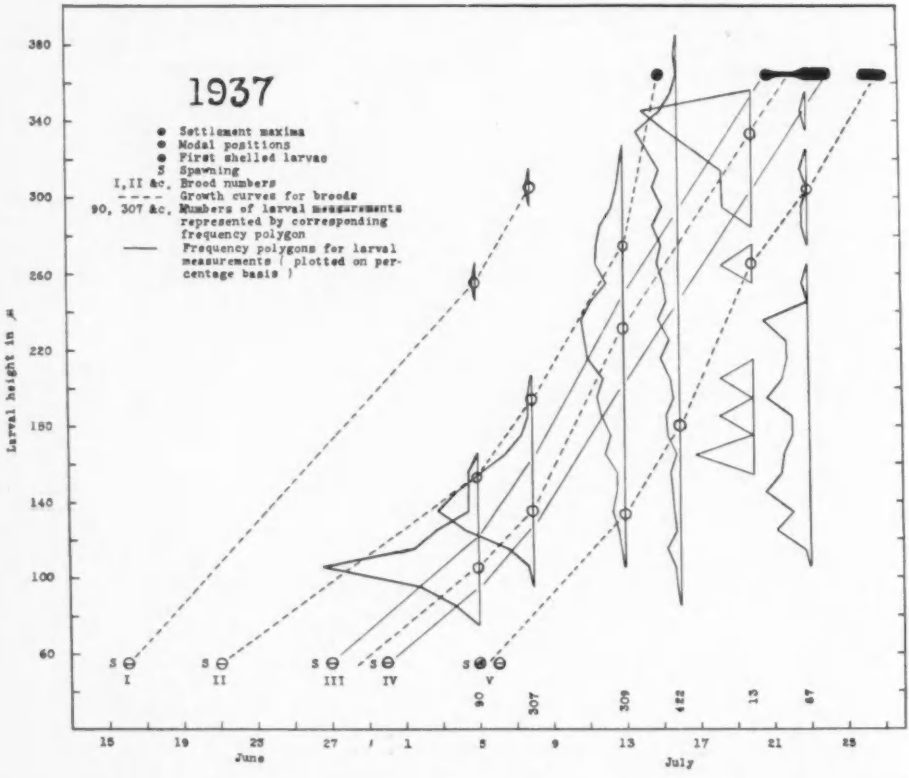


FIGURE 4. Size-frequency distribution of oyster larvae at different times in 1937, plotted on a percentage basis and combined with spawning and settlement observations.

struction of the growth curve for brood V of 1937, for instance, was partly conjectural. Such license was taken because of the apparent soundness of the location of other curves like that for broods III and IV of 1937.

RELATION OF SPAWNING TO TEMPERATURE

An examination of figure 2 shows that in all cases spawning was preceded by sudden rises of water temperature. On June 24 and July 3 of 1936 and on June 20 of 1937, spawning took place but the maximum temperatures recorded on

these days were all below the spawning threshold. The importance of these observations is not to be overlooked. Apparently the suddenness of the rise is as significant a factor in the requirements for spawning as the mere attainment of any fixed temperature level.

Another interesting fact is that June 10, 1936, and June 8, 1937, were the first dates of the respective years when water temperatures rose above the spawn-

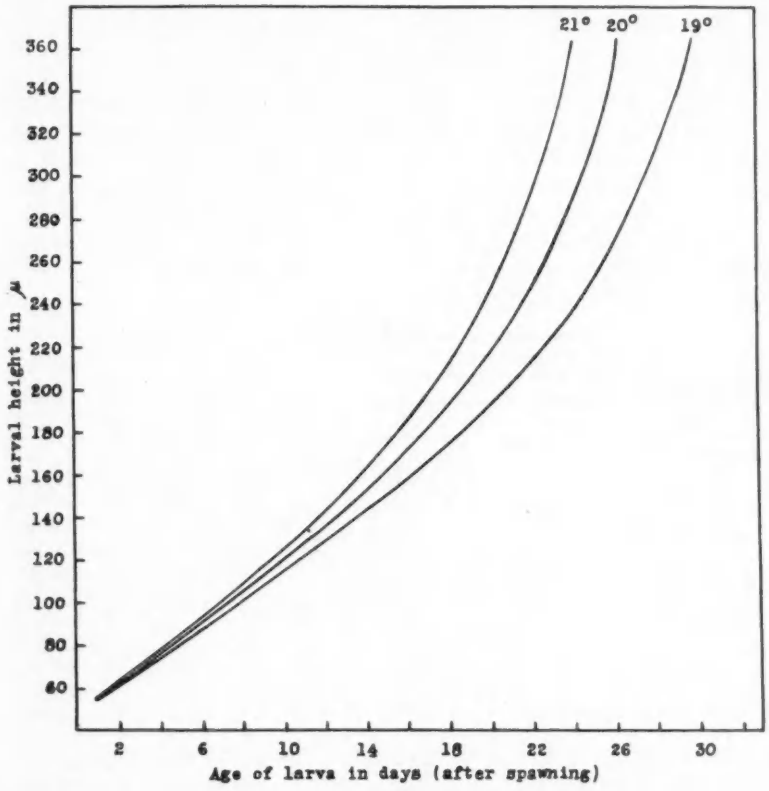


FIGURE 5. Approximate growth curves for Bideford river oyster larvae at constant surface temperatures of 19, 20 and 21°C.

ing threshold. On neither date, however, was there any evidence of spawning and studies of gonads showed that the oysters were immature (tables I and II). According to Hopkins (1931) this is not an uncommon occurrence off the coast of Texas. The conclusion is that temperature can be regarded as the controlling factor for spawning only when the oysters are "ripe".

Clearly temperature records alone cannot be depended upon to determine spawning dates. They must be supplemented with studies of gonads or plankton catches.

Relation of Spawning to Lunar Cycles

No relation could be found between spawning dates in Bideford river and lunar cycles. This is to be contrasted with the findings of Prytherch (1928) and others, who have shown that in some districts where there are extensive tidal flats, variations in water temperature, and hence oyster spawnings, are directly related to tidal phases and thus indirectly to lunar cycles. Day to day changes in the amount of incident solar radiation and air temperatures determine the water temperatures in Bideford river.

Relation of Larval Growth to Temperature

Table IV lists the increments between successive points on the growth curves of figures 3 and 4, along with the corresponding time intervals and average water temperature for each interval. From these data approximate curves for larval growth at constant temperatures of 19, 20 and 21°C. were developed in figure 5. These curves were obtained by piecing together parts derived from table IV and smoothing the result. Owing to the method of construction, all the errors in locating the different segments of the curves are cumulative. The curves are therefore necessarily only approximate.

Table IV. Growth increments of broods at various temperatures

Increment (μ)	Time interval (days)	Average temperature (°C.)	Brood Number	Year	Dates
At about 19°C,					
55–210	20	18.9	II	1936	June 25–July 15
55–255	19	19.5	I	1937	June 16–July 5
105–135	5	18.8	IV	1937	July 15–July 20
125–155	5	18.8	III	1937	July 15–July 20
165–365	19	19.0	I	1937	June 26–July 15
210–235	5	18.8	II	1937	June 15–July 20
At about 20°C.					
55–105	8	20.4	V	1936	July 21–July 29
55–125	11	20.3	III	1936	July 4 –July 15
55–155	14	19.9	II	1937	June 21–July 5
105–120	3	20.5	V	1936	July 29–Aug. 1
135–155	4	20.5	IV	1936	July 20–July 24
155–185	4	20.5	III	1936	July 20–July 24
155–200	5	20.1	IV	1936	July 24–July 29
185–255	5	20.1	III	1936	July 24–July 29
205–260	3	20.4	VI & VII	1936	Aug. 17–Aug. 20
200–365	3	20.5	IV	1936	July 29–Aug. 1
255–365	1	20.5	III	1936	July 29–July 30
At about 21°C.					
55–105	7	21.3	III & IV	1937	June 28–July 5
55–105	6	21.1	IV	1936	July 9 –July 15
55–160	12	21.2	VI & VII	1936	July 31–Aug. 12
120–155	3	20.9	V	1936	Aug. 1 –Aug. 4
135–180	3	20.8	V	1937	July 13–July 16
155–205	5	21.3	V	1936	Aug. 7 –Aug. 12
160–205	5	20.9	VI & VII	1936	Aug. 12–Aug. 17
180–265	4	21.3	V	1937	July 16–July 20
275–365	2	21.1	II	1937	July 13–July 15
285–365	3	20.9	V	1936	Aug. 12–Aug. 15

From figure 5 it is clear that in every case the growth rate increases towards the end of the larval period, there being no suggestion of the sigmoidal nature of Nelson's curve (1923). Since so many measurements were involved in the present study the writer feels that for Bideford river larvae at least, the simpler curve is truly representative of growth conditions.

Relation of Length of Larval Period to Temperature

The length of the larval period in Bideford river is clearly determined by water temperature. In table V are the data pertinent to this relationship and in figure 6 they are graphically represented. The uniformity of water temperatures from surface to bottom in Bideford river (figure 2) and their slight fluctuations with tidal changes (Needler unpub.) present ideal conditions for the study of temperature effects on the larval period.

Table V. Summary of brood histories

Brood number	Date of spawning	Date of settlement	Length* of larval period (days)	Average temperature during larval period (°C.)	
				Surface only	Both surface and bottom
1936					
I	June 14	July 14–15 (approximate)	30–31	18.7	
II	June 24	.....	.....	....	
III	July 3	July 29–30	26–27	20.0	
IV	July 8	August 1–2	24–25	20.3	
V	July 20	August 14–15	25½	20.8	
VI	July 28	August 23±1	25±1	20.6	
VII	August 2	August 27±1	25±1	20.1	
1937					
I	June 15	.....	.....	....	....
II	June 20	July 15	24	20.5	20.0
III	June 26	July 21	25	21.3	20.8
IV	June 29	July 23–24	24½	21.7	21.3
V	July 4 & 5	July 26–28	23–24	22.2	22.0

*The day of settlement is not included in the free-swimming period.

Since in 1936 only surface readings were made, the average temperatures during the larval periods for both years in one column of table V are calculated from surface readings only. On this comparable basis a curve is drawn in figure 6. A truer guide to the position for the curve is probably to be had from the second set of 1937 readings of both surface and bottom temperatures.

In the literature there are frequent statements to the effect that temperature determines the length of the larval period, yet seldom is it clearly demonstrated. Prytherch's data (Galtsoff, Prytherch and McMillin 1930) also plotted in figure 6, show that conditions obtaining in Connecticut waters are in some respects similar to those in Bideford river.

Because of the scattering of the observations over such a limited range of temperature there is some doubt as to where the curves should be drawn. The points for both districts however fit a hyperbola. The effect of temperature on growth in oyster larvae is therefore similar to that in other invertebrates.

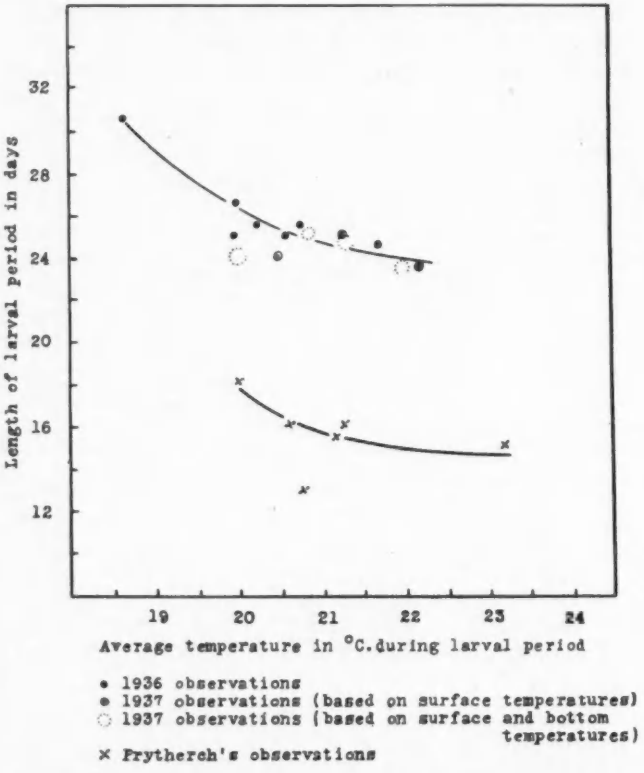


FIGURE 6. The effect of temperature on the length of the larval period.

RELATION OF LENGTH OF LARVAL PERIOD TO SALINITY

While Prytherch (1934) states that when salinities are lower than usual in Long Island sound the larval periods are shorter, and *vice versa*, such a condition has not been found in Bideford river, apparently because the salinity variation is so slight.

PREDICTION OF SETTLEMENT MAXIMA

In 1937 there was, as a background, only the knowledge obtained in 1936 as to growth rates of the larvae. The size-frequencies of the larvae taken on July 13 permitted the prediction of sets for July 15-17 and 19-23. The records (figure 2) show that these were justified for there were heavy spatfalls on July 15, 21 and

23-24. With the data for the two years available more precise forecasts were possible and made in 1938 both for Bideford river and the Bras d'Or lakes. The advantages of reliable spatfall prediction over a week in advance are apparent to those acquainted with the practices of oyster farming.

SUMMARY AND CONCLUSIONS

Once oysters are ripe, spawning occurs with rising temperatures which may or may not reach 20°C. Temperature records are of assistance but cannot be relied upon alone in determining spawning dates.

No relation could be found between lunar cycles and spawning dates for Bideford river.

In making size-frequency distributional studies of oyster larvae, heights are more reliable measurements than lengths.

The mean height of mature oyster larvae in Bideford river is 365μ. This size seems relatively constant and once the larvae reach it they apparently settle without delay. The times of spatfall maxima depend on spawning dates and subsequent water temperatures, not on lunar cycles.

The growth rate of larvae and the length of the larval period are clearly determined by temperature, it requiring approximately 24, 26 and 30 days to reach maturity at constant temperatures of 21, 20 and 19°C. respectively.

The growth curves for larvae are not sigmoidal, for during the last few days the growth rate increases rapidly.

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Order of Appearance of Scales in Speckled Trout

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ABSTRACT

In *Salvelinus fontinalis* two scale papillae develop on the lateral line over each myotome. They appear as far back as the adipose fin and independently on the dorsal line anterior to the adipose fin. The scale pattern develops by extensions obliquely forward from the primary papillae. Development is more rapid in the posterior region. Chief variations are bifurcation of rows and extension of rows posteriorly.

INTRODUCTION

Study of teleost scales has proved to be a valuable source of information on the life history of fish. Yet few studies have been recorded concerning the order of appearance of scales on the body. Some observations on this order in the speckled trout of eastern North America, *Salvelinus fontinalis*, are offered here.

Klaatsch (1890) was the first to call attention to the order in the appearance of scales. Thomson (1904) summarized most of the literature on teleost scales appearing up to that time. Later Huntsman (1918) dealt with the order of scale appearance in the herring, while Parrott (1934) and Neave (1936) recorded similar observations on species of *Salmo*. Van Oosten (1928) mentions one phase of the subject.

MATERIAL AND METHODS

The specimens used in the present study were collected by the investigators of the Margaree Salmon Investigation in Cape Breton, Nova Scotia, in the summers of 1935, 1936 and 1937. The series included young trout from several streams, ranging from 2.6 to 5.9 cm. in length; it was not possible at the time to obtain a complete series from a single locality. Specimens were preserved in 10% formalin and later measured for total length (tip of nose to end of caudal fin). In preparing the specimen for study the skin was removed from the left side of the body, the pigment carefully scraped from the dermis and the mucus removed from the epidermis. The cleaned skin was mounted flat on a glass slide with albumen fixative. When studying the skins it was found that slight moistening and illumination with a green light were aids to clearness.

J. Fish. Res. Bd. Can. 4 (4), 1939.

As the skins were studied drawings were made of the scale distribution. Squared paper and a Whipple disc in the ocular of the microscope were used to insure correct proportion in the drawings.

TIME REQUIRED FOR SCALES TO DEVELOP

Parrott (1934) and Neave (1936) found the first appearance of papillae, on various species of *Salmo*, to be at a length between 2.5 and 3.0 cm. In the smallest speckled trout studied, 2.6 cm. long, taken on June 14, 1937, small patches of dense tissue occurred along most of the length of the lateral line. On a 3.6 cm. specimen papillae appeared dorsally and ventrally to those on the lateral line. On a 4.0 cm. specimen there were up to seven horizontal rows of papillae dorsal to the lateral line. By this time scales were formed on the earlier papillae and showed sufficient growth to cause some over-lapping near the lateral line.

A specimen taken on September 4, 1936, 5.9 cm. long, had the body entirely covered with scales, except for a small area about the bases of the pectoral fins.

Since hatching occurs about February, the age of the smallest trout studied, on which scale development was at a very early stage, would be about four months. Three to four months more seem to be required for the scales to spread over the entire body with the exception of the area mentioned. Considerable individual variation is shown in the rate of development on trout even from the same location.

DISTRIBUTION OF EARLY SCALE PAPILLAE

Relation to Metamerism

Klaatsch (1890) failed to find for the brown trout any correspondence of the number of scales to myocommata, which would mean to myomeres, but Ryder (1892) found for teleosts in general that such a correspondence existed, the number of oblique rows of scales corresponding to the number of somites, or being repeated as multiples of the somites, or being reduced below the number of somites. Subsequent investigators (Huntsman 1918, for the herring, Van Oosten 1928, for the cisco) have corroborated Ryder. The speckled trout shows this correspondence, the details of which follow.

In a 3.2 cm. specimen, along the lateral line, in the area below the adipose fin, the largest papillae were located immediately behind the point at which the myocommata met the lateral line. A smaller papilla appeared in each myotome just anterior to the posterior myocomma of the myomere (figure 1A). Both large and small papillae would seem to be the "primary papillae" of Neave. The relation of scales to somites was not directly evident away from the lateral line.

Places of First Appearance

Since the first statement on the subject by Klaatsch (1890) that in the brown trout the scales first appear in the anterior and middle part of the trunk in the vicinity of the lateral line, little attention has been given to the matter of places of origin. Huntsman (1918), for the herring, found three such places on each side,

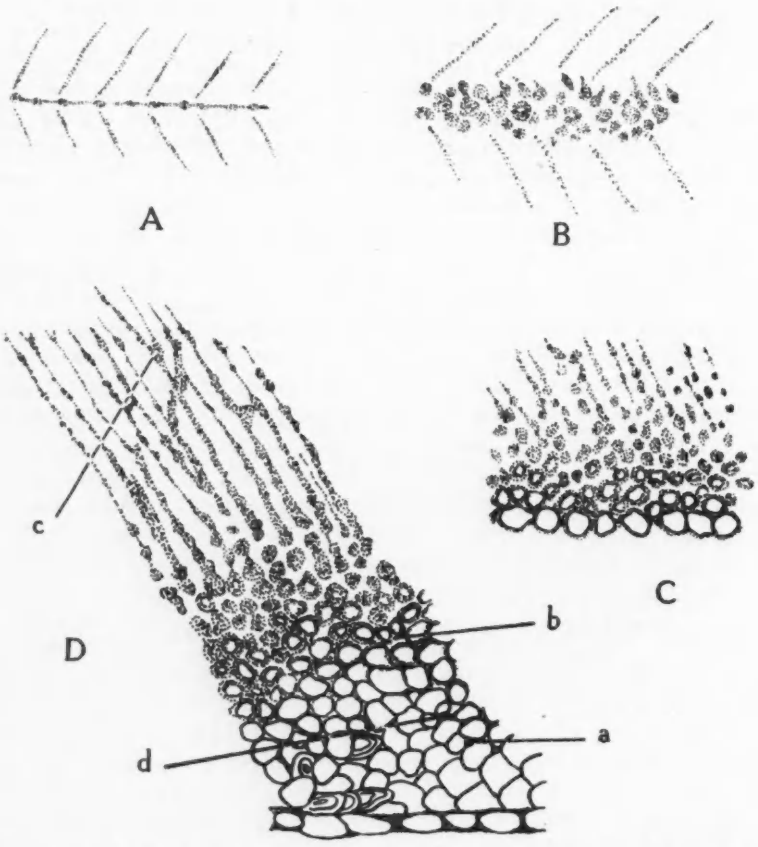

FIGURE 1. A,—lateral line region below adipose fin of a 3.2 cm. trout showing relation of primary scale papillae to myocommata (oblique lines). B,—same region on a 3.6 cm. trout showing relation of secondary papillae to primary lateral line papillae (note elongation of some secondary papillae in a direction obliquely forward). C,—area from region below adipose fin of a 4.0 cm. trout showing oblique rows of scale papillae and lines of dense tissue dorsal to lateral line scales. D,—same as C but from a 5.2 cm. trout showing bifurcation of outgrowths at a, b and c, the last point two-thirds of the way up from the lateral to mid-dorsal lines, also two small papillae side by side in a single outgrowth at d (scales are largely removed and in lateral line and first few rows above it scale pockets rather than scales are shown).

two in relation to the lateral line, one at the anterior end and the other between anal and caudal fins, the third being along the ventral side of the trunk between the pectoral and pelvic fins. For the genus *Salmo*, later investigators agree that the scales first appear along the lateral line. Parrott (1934) found in *Salmo trutta* the scale papillae appear first along the lateral line slightly posterior to the dorsal fin, while Neave (1936) states for several species that they appear nearly simultaneously along the anterior and middle regions of the lateral line, and in addition, at a later date, at a place on the mid-dorsal line a little behind the supra-temporal canal.

In the speckled trout, as judged from the smallest specimens studied, the papillae appear nearly simultaneously along the lateral line, except at the extreme posterior end. There was no indication of a centre of origin in the vicinity of the supra-temporal canal.

A 5.2 cm. trout showed what seemed to be an independent place of origin near the mid-dorsal line, between dorsal and adipose fins. A few papillae could be seen in this region, though the precise nature of their arrangement could not be determined. This apparently separate point of origin was confirmed by the condition in a 5.9 cm. fish, in which the scales near the dorsal line were not quite as large as those near the lateral line but considerably more developed than those about three-quarters of the way up from the lateral line.

MANNER OF SPREADING OVER THE BODY

Investigators previous to Neave (1936) state that development spreads over the body from the places of origin, but, with the exception of Ryder (1892), who says that the scales are arranged in oblique rows, fail to make mention of the precise manner in which spreading occurs. The pattern in which scales spread over the body of the speckled trout is described below.

Development spreads over the body in rows extending obliquely forward from the primary lateral line papillae. In the early stages these rows are hardly distinguishable as such. In a 3.6 cm. specimen, in the region below the adipose fin, there are, in many instances, two papillae in the outgrowths from each lateral line papilla, both dorsally and ventrally. The first papilla in each outgrowth lies in the angle between two of the lateral line papillae. The second lies directly opposite the next primary papilla anteriorly. The arrangement, at first glance, appears to be that of a rosette of six papillae around a primary papilla. Many of the secondary papillae showed a slight elongation in an obliquely forward direction, apparently the direction in which growth proceeds (figure 1B). The lines of outgrowth crossed the direction of the myocommata nearly at right angles. Neave (1936) found similar lines of outgrowth in the brown trout, but does not describe their manner of extension.

In a 4.0 cm. trout development was farther advanced in the posterior region and extended one-third of the way to the mid-dorsal line. The lines of outgrowth contained at most nine distinct scales and papillae. These were progressively smaller outwards, and beyond the last papilla was to be seen a thread of dense tissue extend-

ing about 0.1 mm. (figure 1C). In a specimen 4.9 cm. long there were at most ten distinct scales and papillae in an outgrowth, but the smallest were over twice the diameter of those in the previous specimen. The outgrowths beyond distinct papillae extended a distance of 1 to 2 mm. and were 0.1 mm. wide. In a specimen 5.2 cm. long the outgrowths reached two-thirds of the way up from the lateral line, but scales or papillae were present over only slightly more than half of the total length of each outgrowth. As development proceeds these outgrowths elongate more rapidly than papillae are formed (figure 1D).

The oblique arrangement of the scales was evident on the largest specimen studied, length 5.9 cm. The smallest scales in the region below the adipose fin, as already mentioned, were about three-quarters of the way up from the lateral line, indicating that the scales from the centre of origin in the dorsal region had spread downwards this far. Scales from the region below and anterior to the dorsal fin were smaller and had fewer circuli than those from the region below and posterior to the dorsal fin.

Development below the lateral line is similar to that above, the outgrowths spreading obliquely forward and downward from the lateral line. It is slightly slower than development above the lateral line, as recorded by Parrott (1934) for the brown trout.

VARIATIONS IN THE ABOVE ORDER

Bifurcation of the lines of outgrowth appears to be quite frequent in the speckled trout. Neave (1936) found it to be exceptional in the brown trout. While bifurcation occurred in quite early stages of development, it was more noticeable where the lines of outgrowth had proceeded for some distance. Some lines bifurcated more than once (figure 1D), resulting, over limited areas, in half again as many scales in horizontal rows away from the lateral line as occurred in the lateral line itself. In one specimen, length 3.8 cm., at four scales out from the lateral line there were ten lines of growth arising from six lateral line scales; in another, length 4.9 cm., at eight scales out there were ten lines of growth arising from six lateral line scales. In a 5.2 cm. trout branching occurred more than half way out to the mid-dorsal line (figure 1D). At the edge of the area of development there were twelve lines of growth arising from six lateral line scales. When a line bifurcates the two new lines proceed parallel and adjacent to each other. There appears to be no particular order in the point or extent of branching.

Occasionally two small papillae occur side by side in a line of growth, as though a single papilla had divided to form two. This might be a case of bifurcation where one branch is checked immediately (figure 1D).

Frequently the direction of outgrowth is obliquely posterior, especially below the lateral line (figure 2). Neave (1936) mentions a similar occurrence in the brown trout. While lines of papillae running obliquely posteriorly might be merely owing to the order in which the papillae are placed, at the ends of most rows interpreted as outgrowths there are dense lines of tissue pushing out posteriorly, similar to the lines of tissue at the ends of the typical lines of outgrowth. In most cases,

but not in all, a series of papillae and scales can be traced from such a posteriorly growing line back to the lateral line. Where lines of papillae running obliquely forward meet lines proceeding obliquely backward papillae occur at the point where the two lines meet. Beyond the point of meeting there are either papillae, or the dense lines of tissue preceding formation of papillae, continuing the direction of the forward-growing lines. Where lines of papillae growing backward meet lines growing forward, which already extend some distance beyond the point of meeting, there are few or no papillae beyond the point of meeting in the row extending backward.

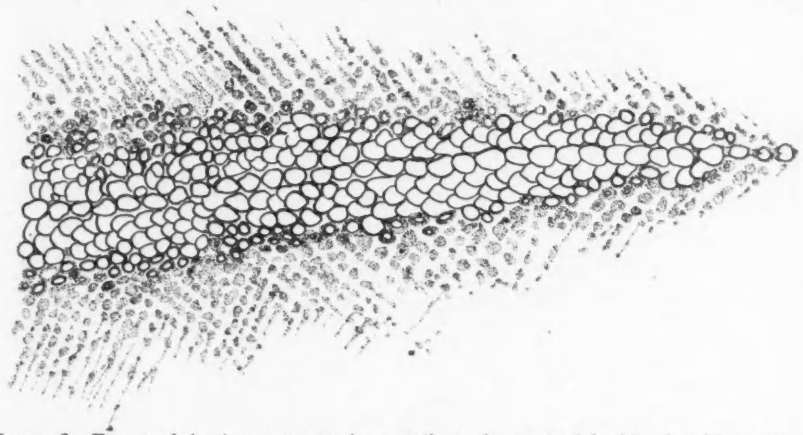

FIGURE 2. Extent of development posterior to adipose fin on the left side of a 4.9 cm. trout, showing scale pockets, scale papillae and lines of dense tissue, also rows extending obliquely posteriorly below the lateral line.

Occasionally a scale appears between and slightly dorsal to two lateral line scales (figure 2), which seems to correspond to those described by Neave (1936) as arising from unlinked neuromasts.

CONCLUSIONS

The first appearance of scale papillae on speckled trout occurs early in the first summer, about four months after hatching, when the fish are 2.5 to 3.0 cm. long. Approximately three months, or until the fish is about 6.0 cm. long, is required for the scales to spread over the entire body, except for an area about the pectoral fins, which does not become scaled until a later date.

Along the lateral line there are two papillae superimposed on each subjacent myomere. The anterior papilla develops first but the posterior one attains the same size as the anterior at an early stage.

The first appearance of scale papillae is on the lateral line, almost simultaneously from the anterior end to the region below the adipose fin. From the latter region development proceeds most rapidly. Another place of origin, of secondary importance, is along the mid-dorsal line slightly anterior to the adipose fin.

From the place of origin on the lateral line development proceeds by outgrowths from the primary papillae which grow obliquely forward, nearly at right angles to the direction of the myocommata. Scale papillae are formed along these outgrowths. Development is most rapid in the area below the adipose fin and slowest below the dorsal fin.

Bifurcation of the outgrowths occurs quite frequently and may result in more scales in a horizontal row away from the lateral line than in the lateral line itself.

Lines of outgrowth may develop obliquely posteriorly, at right angles to the usual direction. Such lines are checked on coming up against a normal line of outgrowth. They occur quite frequently below the lateral line but seldom above.

Scales not in the regular series may occur in or near the lateral line.

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